Status Page

PROTOCOL **11-477**

Closed to New Accrual

Closure Effective Date: 01/15/2016
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

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

Office for Human Research Studies

Protocol Front Sheet

DFCI Protocol No.: 11-477

1. PROTOCOL TITLE AND VERSION

Title: Randomized phase II study of fulvestrant with or without ganetespib in patients with hormone receptor-positive, metastatic breast cancer
Protocol Version No./ Date: 12/14/2015 **Sponsor Study Number:** N/A

2. DF/HCC STUDY CONTACT INFORMATION

Primary Study Contact: Danielle Gore **Email:** dgore2partners.org **Phone:** 617-632-4870

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

Overall PI:	Nancy Lin, MD	**Phone:**	617-632-2335	**Institution(s):**	DFCI
Site Responsible PI:	Douglas Weckstein, MD	**Phone:**	603.622.6484	**Institution(s):**	NHOH

3. DRUG / DEVICE INFORMATION N/A:

☒ **Drug(s), Biologic(s)**: Ganetespib (STA-9090)
Provided by: Synta Pharmaceuticals
IND Exempt: ☐ -or-
IND#: 115026 **Holder Type:** DF/HCC Investigator
IND Holder Name: Nancy Lin, MD

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

4. PROTOCOL COORDINATION, FUNDING, PHASE, MODE, TYPE ETC.

Regulatory Sponsor:
DF/HCC Investigator Nancy Lin, MD

Cancer Related: Yes If yes:
Primary Disease Program:
Breast Cancer
or
Primary Discipline Based Program:
[pull down]

Funding/Support (check all that apply)**:**
☒ Industry: Synta Pharmaceuticals
☐ Federal Organization:
Grant #:
☐ Internal Funding:
☐ Non-Federal:
☒ Other: Komen Grant #KG110450

CTEP Study: [pull down]

Phase: Phase 2

Multi-Center (i.e., non-DF/HCC site participation)**:**
Yes

Protocol Type: Therapeutic - Continuation
If Ancillary, provide parent protocol #:

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

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

Total Study-Wide Enrollment Goal: 71 **Greater than 25% of the overall study accrual will be at DF/HCC:** ☒ Yes ☐ No
Total DF/HCC Estimated Enrollment Goal: 56 **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. DF/HCC PARTICIPANTS UNDER DFCI IRB (check all that apply)

☐ Beth Israel Deaconess Medical Center (BIDMC)
☐ Beth Israel Deaconess Medical Center – Needham (BIDMC-Needham)
☐ Boston Children's Hospital (BCH)
☒ Brigham and Women's Hospital (BWH)
☒ Dana-Farber Cancer Institute (DFCI)
☐ Dana-Farber/New Hampshire Oncology-Hematology (DFCI @ NHOH)
☐ DF/BWCC in Clinical Affiliation with South Shore Hospital (DFCI @ SSH)
☐ Dana-Farber at Milford Regional Cancer Center (DFCI @ MRCC)
☐ Dana-Farber at Steward St. Elizabeth's Medical Center (DFCI @ SEMC)
☐ Massachusetts General Hospital (MGH)
☐ Mass General/North Shore Cancer Center (MGH @ NSCC)
☐ Mass General at Emerson Hospital – Bethke (MGH @ EH)
☐ New England Cancer Specialists (NECS)

7. NON-DF/HCC PARTICIPANTS UNDER DFCI IRB (check all that apply)

☐ Cape Cod Healthcare (CCH)
☐ Lowell General Hospital (LGH)
☒ New Hampshire Oncology-Hematology-P.A. (NHOH)
☐ Newton-Wellesley Hospital (NWH)
☐ Broad Institute
☐ Lawrence & Memorial Cancer Center in affiliation with Dana-Farber Community Cancer Care (LMCCC)

8. DF/HCC INITIATED STUDIES ONLY - INSTITUTIONAL PARTICIPANTS UNDER OTHER IRB (N/A:)

DF/HCC Multi-Center Protocols: (list institution/location)
University of North Carolina at Chapel Hill, NC
Chapel Hill

DF/PCC Network Affiliates: (list institution/location)

Protocol Number: 11-477

Approval Date: 02/28/12 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 05/18/12 (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
6/1/12	DFCI Nursing Protocol Education Sheet Updated	N/A	-
6/1/12	Alert Page Updated Due to Amendment #4	5/24/12	-
09/24/12	Front Sheet replaced due to Amendment #5	09/21/12	N/A
09/28/12	Temporary Closure to New Accrual: due to Administrative HOLD per DF/HCC DSMC (effective date: 09/20/12 ; Amendment # 6)	09/24/12	N/A
10/11/12	Alert Page, Protocol and Front Sheet replaced due to Amendment #7	10/04/12	N/A
10/16/12	Re-Open to accrual; Amendment #8 (Effective 10/10/12)	10/12/12	N/A
11/08/12	Protocol and Front Sheet replaced due to Amendment #9	10/26/12	N/A
01/30/13	Front Sheet replaced due to Amendment #10	01/29/13	n/a
02/22/13	Study renewal/ Consent Form footer replaced due to Continuing Review #1	02/14/13	N/A
02/28/13	Protocol, Consent Form and Front Sheet replaced, Pharmacy Manual added due to Amendment #11	02/19/13	02/28/13
03/25/13	Correction: alert page removed (AM #11)	n/a	n/a
03/26/13	Front Sheet replaced due to Amendment #12	03/25/13	n/a
04/02/13	Protocol and Front Sheet replaced due to Amendment #13	04/01/13	N/A
04/26/13	Front Sheet replaced due to Amendment #14	04/26/13	N/A
06/03/13	Protocol, Consent Form and Front Sheet replaced due to Amendment #15	05/30/13	06/03/13
07/23/13	Protocol and Front Sheet replaced; Study Medication Preparation & Administration (300 mg) and Recruitment Materials added due to Amendment #16	07/11/13	N/A
08/05/13	Alert Page added due to Amendment #17	07/22/13	N/A
08/28/13	No changes to online documents; Amendment #19	08/16/13	N/A
09/11/13	BIDMC added as participating site; Consent Form and Front Sheet replaced due to Amendment #18	08/23/13	09/05/13
09/17/13	Site Specific Closure: temporary closing BIDMC site to new accrual; remains open at DFCI, DFCI at FH (effective 09/16/13; Amendment #20)	09/17/13	n/a
10/02/13	Re-open to accrual at BIDMC; Amendment #22 (Effective 09/24/13)	09/25/13	N/A
10/02/13	Consent Form and Front Sheet replaced due to Amendment #21	10/02/13	10/02/13
10/03/13	Consent Form, Protocol and Front Sheet replaced due to Amendment #23	09/30/13	10/02/13
10/09/13	Correction: Consent Form replaced due to incorrect version previously provided w/ Am #23	09/30/13	10/08/13
10/17/13	Front Sheet replaced due to Amendment #25	10/15/13	n/a
10/24/13	Protocol and Nursing PES replaced, Alert Page removed due to Amendment #24	10/08/13	N/A
11/13/13	Protocol, Consent Form and Front Sheet replaced due to Amendment #26	11/07/13	11/13/13

11/26/13	Correction: Consent Form replaced due to incorrect version previously provided w/ Am #26	11/07/13	11/26/13
01/27/14	Consent Form replaced due to Continuing Review #2	01/23/14	01/24/14
02/12/14	Alert Page added due to Amendment #27	01/23/14	N/A
03/18/14	Consent Form, Protocol and Front Sheet replaced due to Amendment #28 (Note: re-consent required)	02/25/14	03/18/14
04/02/14	Protocol, Pharmacy Manual-300 mg and Front Sheet replaced due to Amendment #29	03/24/14	N/A
04/04/14	Correction AM #29: Alert Page removed	N/A	N/A
04/04/14	Update: PES replaced	N/A	N/A
04/15/14	Alert Page added due to Amendment #30	04/09/14	n/a
05/14/14	Alert Page and Front Sheet replaced due to Amendment #31	05/02/14	N/A
06/03/14	Front Sheet replaced due to Amendment #32	06/02/14	n/a
07/25/14	New Hampshire Oncology – Hematology site closed to new accrual; All other sites remain open to accrual; Amendment # 33 (Effective 06/30/14)	07/01/14	N/A
08/14/14	Administrative Update #1: Pharmacy manuals 400mg and 300mg replaced with Pharmacy Manual	N/A	N/A
10/07/14	Protocol, Front Sheet , Nursing PES replaced due to Amendment #34	09/30/14	N/A
10/14/14	Correction Amendment #34: Alert Page removed	N/A	N/A
11/11/14	BIDMC removed from the study. Consent Form and Front Sheet replaced due to Amendment #35	11/10/14	N/A
01/05/14	Consent Form replaced due to Amendment #36	12/22/14	12/23/14
01/22/15	Study renewal / Consent Form footer replaced due to Continuing Review #3	01/08/15	N/A
05/18/15	Protocol, Consent Form and Front Sheet replaced due to Amendment #37	05/15/15	05/18/15
Date Posted	**Revised Sections**	**IRB Approval Date**	**OnCore Version Date**
10/20/15	Front Sheet replaced due to Amendment #38	10/16/15	N/A
12/28/15	Study renewal / Consent Form footer replace due to Continuing Review #4	12/17/15	12/18/15
01/06/16	Protocol, Consent Form and Front Sheet replaced due to Amendment #39	12/29/15	01/05/16
01/15/16	Permanent Closure to New Accrual: Slow accrual (effective date: 01/15/16; Amendment #40)	01/08/16	N/A
Date Posted	**Revised Sections**	**Approved Date**	**Version Date (OnCore)**
12/16/16	Study renewal/Consent Form footer replaced due to Continuing Review #5	12/15/16	12/16/16
12/14/2017	Study renewal/ Consent form footers replaced per Continuing Review #6	10/19/2017	11/05/2017

Protocol Version Date: December 14, 2015

Local Protocol #: 11-477

Title: Randomized phase II study of fulvestrant with or without ganetespib in patients with hormone receptor-positive, metastatic breast cancer

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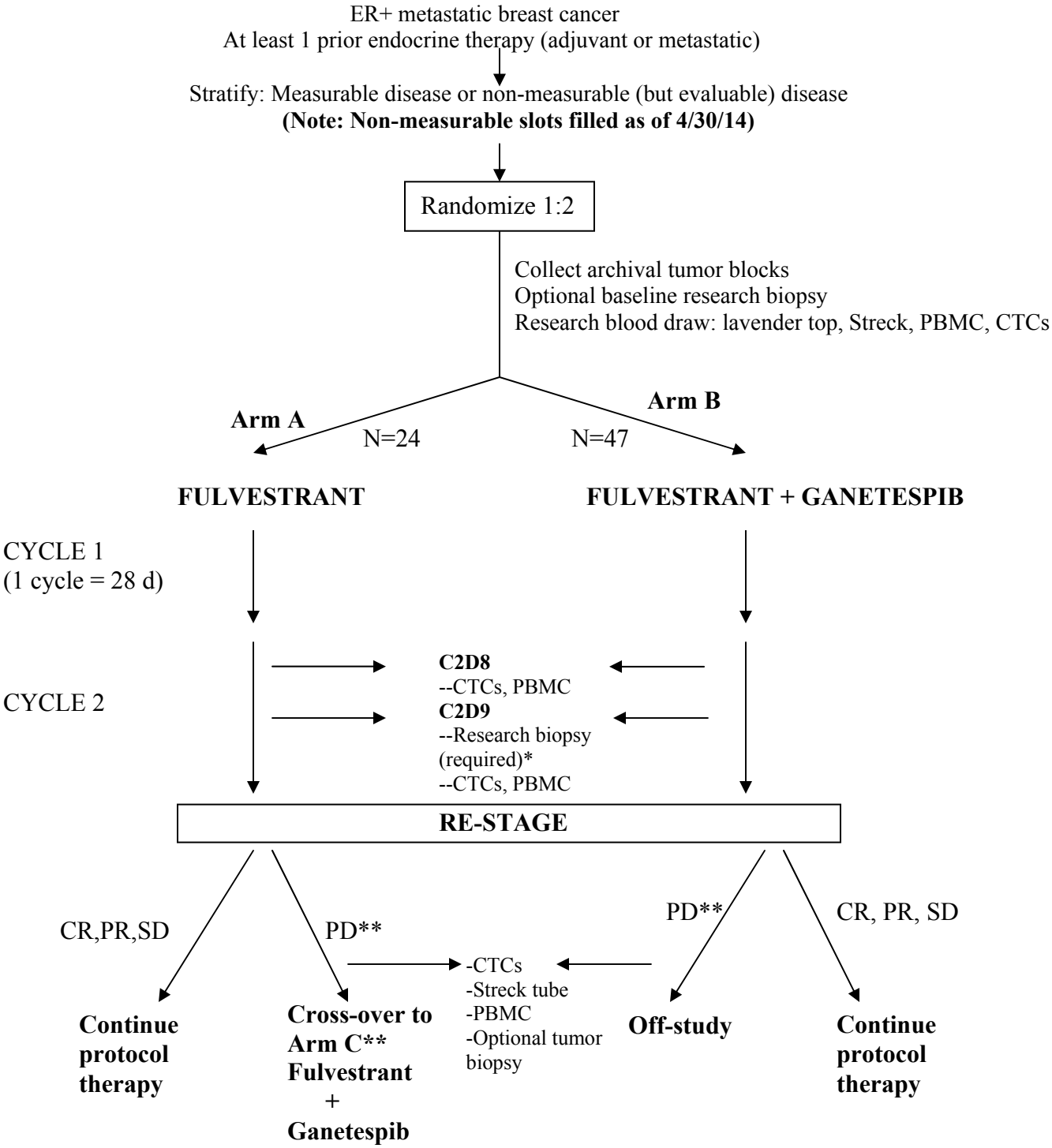
Agent(s): ganetespib – Synta Pharmaceuticals; Fulvestrant (commercial supply)

SCHEMA



*on-study biopsy required for patients with biopsy-accessible disease (Section 3.1.14 and Section 8.1)

**Central confirmation of PD required PRIOR to cross-over from Arm A to Arm C, or off-study for PD (Arm B). Patients starting on Arm A who cross over to Arm C (fulvestrant + ganetespib) will be restaged every 2 cycles and continue on therapy until time of progression. However, patients who have been on-study for >1 year will be restaged every 2-4 cycles. At time of off study, CTCs, CPT tube, and optional tumor biopsy will be collected.

NOTE: Patients who come *off protocol treatment* for a reason other than progression or death should remain on study and continue to be followed per protocol guidelines to assess for progression per Study Calendar D. See Section 5.6 and 5.8 for details.

Table of Contents

1. OBJECTIVES

1.1 Study Design

This is a randomized phase II trial of fulvestrant alone or in combination with ganetespib (STA-9090) (a novel HSP-90 inhibitor) in patients with hormone receptor-positive, metastatic breast cancer.

1.2 Primary Clinical Objective

The primary objective of this trial is to evaluate the effect of the addition of ganetespib to fulvestrant on progression-free survival (PFS), compared to fulvestrant alone.

1.3 Secondary Clinical Objectives

- To assess the safety and tolerability of ganetespib in combination with fulvestrant
- To describe and compare the objective response rate by RECIST 1.1 between arms, limited to patients with measurable disease at baseline.
- To describe and compare the clinical benefit rate, defined as complete response (CR) + partial response (PR) + stable disease >/= 6 months between arms
- To describe and compare overall survival (OS) between arms

1.4 Correlative Objectives

1.4.1 The primary correlative objective is to test whether high nuclear expression of HSF1 (as assessed on archival breast tumor samples) is associated with PFS, separately in the fulvestrant only arm and the combination arm.

For this analysis, we will assay existing archival breast tumor specimens. Metastatic specimens will take priority over primary tumor specimens; however both should be collected when available. We hypothesize that high nuclear expression of HSF1 may be associated with worse progression-free survival in patients treated with fulvestrant alone. In patients treated with the combination of fulvestrant + ganetespib, we hypothesize that because high nuclear expression of HSF1 may be indicative of increased dependence on the heat shock network, it may be associated with improved PFS.

1.4.2 All other correlative objectives are exploratory and hypothesis-generating only. They are listed below:

- We will collect cycle 2 biopsies (required in patients with biopsy-accessible disease, see Section 3.1.14 and Section 8.1) to assess the effect of fulvestrant alone versus fulvestrant plus ganetespib within tumor specimens. Using a Nanostring custom code set, we will assess a set of genes regulated by HSF1, the major transcriptional regulator of inducible expression for the entire heat shock network. We will also

assess a panel of estrogen and ER-regulated genes, other downstream targets of Hsp90, and genes thought to play a role in malignant progression/endocrine resistance.

We will compare cycle 2 biopsy specimens between patients treated with fulvestrant alone versus those treated with fulvestrant + ganetespib. We will use a cutoff of 4-fold increase or decrease relative to standard reference genes to characterize the expression of each of the selected genes (upregulated, downregulated, or neutral) for comparison between groups.

- We will tabulate the expression of the same set of genes as specified above (by Nanostring custom gene set), categorized as above (upregulated, downregulated, neutral), in archival specimens (primary and metastatic, as available), cycle 2 tumor biopsies, and optional time-of-progression tumor biopsies, in order to describe the stability of these markers over time

- Using the cycle 2 biopsies (required in patients with biopsy-accessible disease, see Section 3.1.14 and Section 8.1), we will also compare the expression of ER, PR, AR, HSF1, HER2, and EGFR by immunohistochemistry and amplification of EGFR and HER2 using fluorescence in situ hybridization (FISH) to assess the effect of fulvestrant alone versus fulvestrant plus ganetespib within tumor specimens.

  For this analysis, each of the markers will be described as "positive" or "negative". For ER, PR, AR, and EGFR, "positive" will include "high positive", defined as $\geq$10% nuclear staining, and "low positive" will be defined as 1-10% nuclear staining, and "negative" will be defined as no staining or only rare cells positive. For HSF1, "positive" will be defined as 2+ staining, and "low/negative" will include "low-positive" (1+ staining), and true negatives (no staining or rare cells positive) (Santagata et al, manuscript in preparation). For HER2, we will use the standard 0-3+ scale, but will dichotomize with 0, 1+, and 2+ being negative and 3+ positive.

  For this analysis, FISH ratio $\geq$ 2.0 will be considered positive. FISH ratio < 2.0 will be considered negative.

- We will tabulate the expression of ER, PR, AR, HSF1, HER2, and EGFR by immunohistochemistry in archival specimens (primary and metastatic, as available), cycle 2 tumor biopsies, and optional time-of-progression tumor biopsies, in order to describe the stability of these markers over time.

- We will tabulate the amplification status of EGFR and HER2 using fluorescence in situ hybridization (FISH) in archival specimens (primary and metastatic, as available), cycle 2 tumor biopsies, and optional time-of-progression tumor biopsies, in order to describe the stability of these markers over time.

- We will describe ER, PR, AR, and HSF1 expression (using the same cutoffs as above) and describe HER2/EGFR amplification (using the same cutoffs as above) in

circulating tumor cells (CTCs) pre-therapy, Cycle 2 Day 8, Cycle 2 Day 9, and at time of progression, separately by arm of treatment.

- We will explore the relationship between EGFR and/or HER2 amplification in baseline CTCs and PFS, in each arm separately.

- We will compare ER, PR, AR, HSF1 expression, and HER2 and EGFR amplification status on cycle 2 Day 9 on-study biopsy and Cycle 2 Day 9 CTCs.

- We will collect peripheral blood mononuclear cells (PBMC) pre-therapy, Cycle 2 Day 8, Cycle 2 Day 9, and time of progression. Using a Nanostring custom code set, we will assess a set of genes regulated by HSF1, the major transcriptional regulator of inducible expression for the entire heat shock network. We will also assess a panel of estrogen and ER-regulated genes, other downstream targets of Hsp90, and genes thought to play a role in malignant progression/endocrine resistance. We do not expect changes in estrogen and ER-regulated genes or genes thought to play a role in malignant progression/endocrine resistance, but will do this in order to have a consistent assay between PBMC samples and tumor samples as described above. We will describe gene expression over time upon exposure to fulvestrant or the combination of fulvestrant + ganetespib.

- We will describe the frequency of mutations in PIK3CA, ESR1, and other potential genes of interest in archival tumor specimens, cycle 2 Day 9 biopsy specimens, and at the time of progression (when available)

- We will explore the relationship between the presence of a PIK3CA and ESR1 mutations in archival tissue and PFS, in each arm separately

- We will describe mutations in PIK3CA, ESR1, and other potential genes of interest in circulating free DNA (cfDNA) at baseline and time of progression.

- We will explore the relationship between baseline mutations in PIK3CA and ESR1 in circulating cfDNA and PFS, in each arm separately.

- We will tabulate the PIK3CA and ESR1 mutations detected in cfDNA and tumor tissue in order to explore the level of agreement between these two methods

- We will bank any extra materials for future study. We anticipate that this annotated repository will be valuable for future research into the mechanisms of resistance to fulvestrant and to ganetespib.

2. BACKGROUND

2.1 Ganetespib (STA-9090)

Mechanism of Action

Ganetespib (STA-9090) is a novel, synthetic small molecule that inhibits heat shock protein 90 (HSP90), a molecular chaperone protein that modulates cellular homeostasis and responses to environmental stress by regulating post-translational folding of client proteins. Inhibition of HSP90 leads to aberrant protein conformations, which targets most clients for ubiquitination and subsequent degradation via the proteosome. Of note, HSP90 client proteins include the steroid hormone receptors (ER, PR, AR, etc), BCR-ABL, B-RAF, CDK4, c-KIT, EGFR, HER2, IGF-1R, hTERT, Src, PI3K, p53, MEK, PDGFR, VEGFR, and AKT. HSP90 inhibition also has been shown to disrupt signaling through the JAK/STAT pathway.

Preclinical Studies

STA-9090 inhibits HSP90 chaperone activity by binding to its N-terminal ATP pocket. It is a novel compound unrelated to geldanamycin, 17-AAG, or IPI-504.

STA-9090 has cytotoxic activity against a broad range of cell lines derived from hematologic and solid tumors. The IC_{50} values for STA-9090 are in the low nM range and at least 20-fold more potent than 17-AAG. In all but 3 cell lines tested, STA-9090 was more potent than 17-AAG at inducing cell death. In BT-474 breast cancer cell lines, STA-9090 induced significantly more degradation of HER2. A 5-minute exposure to STA-9090 was able to depress HER2 protein levels and maintain this state over the subsequent 3 days.

Animal Studies

In nonclinical studies evaluating absorption, distribution, biotransformation, and elimination, STA-9090 peak and total exposure increased in an approximately dose-proportional manner in rats and cynomolgus monkeys over the dose ranges tested. No significant gender differences were observed in the pharmacokinetics in either species. STA-9090 was highly protein-bound and highly distributed through tissues, with the exception of the central nervous system. Fecal elimination through bile was the major source of excretion. STA-9090 was extensively metabolized in the liver to mainly glucuronide conjugates. STA-9090 appears to be an inhibitor of CYP2C19 and CYP3A4 (midazolam specific), but does not seem to be an inducer of CYP/UDP-UGT enzymes.

Results from safety pharmacology show acceptable cardiac effects, in particular, no evidence of QT prolongation. In genetic toxicity studies, STA-9090 was demonstrated to be non-mutagenic and non-clastogenic.

STA-9090 was highly efficacious in the Daudi Burkitt's non-Hodgkin lymphoma xenograft model. Intravenous (i.v.) bolus dosing via the tail vein at a dose of 25 mg/kg STA-9090 on a

schedule of 1 time per day, 5 times per week (125mg/kg weekly) resulted in significant tumor growth inhibition. Consistent with the increased *in vitro* potency of STA-9090 relative to 17-AAG, a three-fold higher dosage of 75 mg/kg 17-AAG (375 mg/kg weekly) caused only modest tumor growth inhibition in this model.

In conjunction with the above study, the tolerability of STA-9090 was evaluated by measuring cumulative average body weight changes for each treatment group over the course of the study. Both STA-9090 and 17-AAG were well tolerated at these dosages, with only minimal effects on body weights. In this and other studies, 25-35 mg/kg STA-9090 or 75-100 mg/kg 17-AAG dosed 5 times per week (125-175 mg/kg and 375-500 mg/kg weekly, respectively) were the highest dosages that were well tolerated based on body weights in Nude and SCID mice. This was based on no single animal in a treatment group losing ≥20% of its body weight at any point during a study, and no treatment group losing ≥10% cumulative average body weight over the course of a study. However, when dosing only 1, 2 or 3 times per week, even higher doses of STA-9090 were well tolerated.

STA-9090 was also highly efficacious in the SU-DHL-4 GCB-type diffuse large B-cell lymphoma (DLBCL) xenograft model. In this study, 100mg/kg STA-9090 dosed only 2 times per week (200 mg/kg weekly) resulted in significant tumor growth inhibition, with a %T/C value of 2. Again, emphasizing the increased potency of STA-9090, an equivalent dosage of 100 mg/kg 17-AAG (200 mg/kg weekly) was not significantly efficacious, with a %T/C value of 72.

Both drugs had only minimal effects on cumulative average body weights in this study. Treatment with 150 mg/kg STA-9090 dosed only 1 time per week also displayed significant efficacy in this tumor model. The STA-9090 dose response was also examined in the SU-DHL-4 DLBCL xenograft model. Doses of 25, 50, 75 and 100 mg/kg STA-9090 dosed 2 times per week were all highly efficacious in this model, with %T/C values of 26, 4, -90 and -93, respectively. However, doses 6.25 and 12.5 mg/kg STA-9090 failed to show significant efficacy, with %T/C values of 69 and 57, respectively. Seventy five and 100 mg/kg STA-9090 dosed 2 times per week for a total of 3 weeks (150 and 200 mg/kg weekly) resulted in 25% and 50% of the animals in each group being free of tumors by the end of the study, respectively.

A similar STA-9090 dose response was observed in the RPMI 8226 human multiple myeloma xenograft model. Doses of 25, 50, 100 and 150 mg/kg STA-9090 dosed 1 time per week were all efficacious in this model, with %T/C values of 38, 11, -19 and -66, respectively. Importantly, these results illustrate that dosing STA-9090 only once weekly can still result in significant *in vivo* efficacy. It is likely that the STA-9090 dose and dosing schedule necessary to achieve significant efficacy in a xenograft tumor model depends upon the specific HSP90 client proteins that are critical for the proliferation and survival of a particular tumor cell line, as well as the kinetics of HSP90 client protein degradation and re-synthesis after treatment with a HSP90 inhibitor.

Although human cancers are typically characterized by a wide variety of genetic alterations that collectively contribute to the transformed state, a subset of cancers appears to be particularly dependent upon single oncoproteins for their genesis, sustained proliferation and/or survival. In such cancers, inactivation of the critical oncoprotein can cause rapid tumor cell growth arrest,

differentiation or apoptosis. This phenomenon has been termed "oncogene addiction," and has important implications for the development of targeted cancer therapeutics.

Significantly, a number of such addicting oncoproteins are also HSP90 client proteins. Therefore, cancers involving these oncoproteins are particularly promising indications for treatment by HSP90 inhibition. To examine this possibility, STA-9090 was tested in several xenograft tumor models of cancers that are addicted to specific HSP90 client proteins. The best characterized examples of oncogene addiction are in chronic myelogenous leukemia (CML) and B-cell acute lymphoblastic leukemia (B-ALL). The vast majority of CML cases and a subset of B-ALL cases are associated with a chromosomal translocation resulting in expression of the BCR-ABL tyrosine kinase, which is a HSP90 client protein[1]. STA-9090 was highly efficacious in the BCR-ABL+ KU812 CML xenograft model. In this study, 25 mg/kg STA-9090 dosed 5 times per week (125 mg/kg weekly) resulted in dramatic tumor regression, with a %T/C value of -78. In addition, tumors were undetectable in 25% of STA-9090-treated animals by the end of 3 weeks of dosing.

Another example of oncogene addiction is in acute myeloid leukemia (AML) associated with activation of the FLT3 receptor tyrosine kinase, which is a HSP90 client protein. FLT3 activation, most commonly by an internal tandem duplication mutation (termed FLT3-ITD), is the most frequently found genetic alteration associated with AML. STA-9090 was highly efficacious in the FLT3-ITD+ Mv 4-11 AML xenograft model. In this study, 25 mg/kg STA-9090 dosed 5 times per week (125mg/kg weekly) resulted in dramatic tumor regression, with a %T/C value of -94. Tumors were undetectable in 63% of STA-9090 treated animals by the end of 3 weeks of dosing. In contrast, a three-fold higher dosage of 75 mg/kg 17-AAG (375 mg/kg weekly) was substantially less efficacious than STA-9090 in this model, with a %T/C value of 18.

Another example of oncogene addiction is in gastric carcinoma associated with amplification of the c-MET receptor tyrosine kinase, which is a HSP90 client protein. Amplification of the c-MET locus occurs in up to 20% of gastric carcinomas, and inhibition of c-MET activity rapidly induces apoptosis in these cells. STA-9090 was highly efficacious in the c-MET-amplified MKN45 gastric carcinoma xenograft model. In this study, 50 mg/kg STA-9090 dosed 3 times per week (150 mg/kg weekly) resulted in significant tumor growth inhibition, with a %T/C value of 8.

All of the above *in vivo* studies in xenograft tumor models employed STA-9090 solublized in a formulation consisting of 10% DMSO, 18% Cremophor RH40 and 3.6% dextrose in sterile water (DRD). This DRD formulation is well tolerated in mice when i.v. bolus dosed via the tail vein at 5-20 mL/kg. In order to demonstrate that STA-9090 is efficacious when using non-DRD formulations, more representative of that used in the clinic, an *in vivo* efficacy study was conducted with STA-9090 formulated in 45% PEG 400, 55% 50 mM carbonate buffer pH 10.0 in sterile water (45 PEG), which is a formulation that is well tolerated in mice. STA-9090 formulated in 45 PEG was still highly efficacious in the FLT3-ITD+ Mv 4-11 AML xenograft model. In this study, doses of 12.5, 18, 25 and 35 mg/kg STA-9090 dosed 1 time per week were all efficacious, with %T/C values of 42, 30, 11 and -50, respectively. This result indicates that

the *in vivo* activity of STA-9090 in xenograft tumor models is not dependent upon the use of the DRD formulation.

Human Pharmacokinetics

The pharmacokinetics of ganetespib administered at various doses to subjects with solid tumors on a weekly or twice-weekly schedule is under investigation in 2 Phase I trials. Preliminary data and calculated parameters are available in subjects with solid tumors for doses up to 259 mg/m2. Cmax and AUC increase in approximate proportion to dose irrespective of dosing day with virtually identical dose-exposure ratios for Day 1 and Day 15, indicating linear pharmacokinetics. Distribution and elimination phases of ganetespib show maximum concentration at the end of the infusion declining by approximately 10-fold within the first hour and nearly 100-fold within 10 hours following infusion termination. Mean terminal half-lives have ranged from approximately 5.2 to 14 hours. Ganetespib plasma concentrations on Day 1 and 15 are comparable following either once or twice weekly dosing, indicating the lack of drug accumulation. Ganetespib exhibits biphasic pharmacokinetics with concentrations declining by greater than 10-fold within the first few hours following infusion termination. Mean terminal half-lives have ranged from 10.4 to 14.3 hours. STA-9090 plasma concentrations on Day 1 and 15 are comparable, indicating the lack of drug accumulation when dosed either once or twice weekly.

In the dose-escalation 9090-02 trial, blood samples were obtained on C1D1 and C1D15 to quantitate HSP70 RNA levels. Maximum induction occurred in the sample acquired 1.5 to 2 hours after the start of infusion but reached a plateau at low ganetespib levels, suggesting that this may be an overly sensitive biomarker of ganetespib activity.

Drug Interactions

Inhibition and induction potentials of STA-9090 were investigated in human liver microsomes and human hepatocytes. STA-9090 was an inhibitor of CYP2C19 and CYP3A4 (midazolam-specific) but was not an inducer of CYP or UGT isozymes.

At clinically relevant concentrations, STA-9090 inhibited UGT-mediated metabolism to some extent, but not CYP2C9 or CYP3A4-mediated metabolism of potential co-medications in human hepatocytes. STA-9090 did not significantly affect the CYP3A4-mediated metabolism of atorvastatin, dexamethasone, fentanyl, and warfarin, or CYP2C9-mediated metabolism of warfarin. Inhibition of CYP1A2, CYP2B6, CYP2D6, and CYP3A4 (testosterone-specific) were less than 50% at concentrations up to 10 μM.

Based on the Caco-2 permeability assay, STA-9090 is a potential P-gp substrate.
As per Chouinard, et.al, fulvestrant is glucuronidated by UGT1A1, 1A3, 1A4 and 1A8 and ganetespib has been shown to inhibit UGT-mediated metabolism of furosemide (UGT1As); however, laboratory work completed at Synta specifically addressing possible UGT-mediated interactions with ganetespib indicates that an alteration of fulvestrant metabolism by ganetespib is unlikely. Fulvestrant metabolism is unaffected by the presence of ganetespib at ganetespib concentrations of 1, 10, and 20 μM in human hepatocytes *in vitro*. For reference, at 216

mg/m^2 ganetespib, C_{max} is approximately 16 μM. At 150 mg/m^2 it is approximately 11 μM (9090-02)

Synta also addressed DDI potential from the perspective of a possible effect of fulvestrant on ganetespib *in vitro*. There was no alteration of ganetespib metabolism by fulvestrant. Concentrations of 0.3 and 3 μM fulvestrant and 1, 10, and 20 μM of ganetespib were used.

Safety

Data available as of 20 September 2013 for Synta-sponsored studies of single-agent ganetespib in patients with solid tumors and hematologic malignancies.

The MTD for once-weekly dosing in solid tumors was established at 216 mg/m2 ganetespib based on DLTs of asthenia and diarrhea at the highest tested dose level, 259 mg/m2. The recommended once-weekly dose administered 3 consecutive weeks of a 4-week cycle is 200 mg/m2. The recommended dose for twice-weekly dosing (72-hour interval between doses) in patients with solid tumors is 150 mg/m2 administered 3 consecutive weeks of a 4-week cycle and is based on current safety, tolerability, and preliminary activity data. The doses selected for further study in patients with hematologic malignancies were 200 mg/m2 once weekly and 90 mg/m2 twice weekly administered 4 consecutive weeks without a rest week.

Approximately 99% of the patients in the largest pooled data set (single-agent studies, n=378) experienced at least 1 AE; 92% experienced at least 1 treatment-related event. The most frequently reported AEs were related to GI toxicity, and included diarrhea (79%), nausea (42%), decreased appetite (30%), vomiting (25%), constipation (21%), and abdominal pain (19%). Non-GI related events that occurred frequently have included fatigue (54%), headache (19%), and anemia (21%). Two-thirds (66%) of these patients experienced an event that was a Grade ≥3; 31% experienced a Grade ≥3 treatment-related event. Thirty-nine percent experienced at least 1 serious adverse event (SAE); 8% of patients had at least 1 treatment-related SAE.

In the Phase 2b study in patients treated with ganetespib in combination with docetaxel, Study 9090-08, preliminary findings show a similar safety profile with 53% reduction in the incidence of diarrhea. This was due to implementation of antidiarrheal prophylactic medication for patients receiving ganetespib. In the integrated data from patients treated with ganetespib in combination with docetaxel (Studies 9090-07 and 9090-08), 97% of patients experienced at least 1 AE, 77% at least 1 treatment-related event. The most frequently reported AEs were related to GI toxicity and included diarrhea (48%), nausea (25%), decreased appetite (17%), vomiting (14%), constipation (10%), Non-GI related events that occurred frequently include neutropenia (46%), consistent with known toxicity related to docetaxel treatment, fatigue (30%), and anemia (26%).

Of the 223 patients treated with ganetespib in combination with docetaxel, 57% had treatment-emergent AEs that were Grade 3 or Grade 4. Thirty-seven percent had at least 1 SAE and 18%, at least 1 treatment-related SAE. The most common Grade 3 or 4 event in patients receiving the combination treatment was neutropenia (17% and 24%, respectively). In patients treated with docetaxel only, 19% of patients experienced a Grade 3 event of neutropenia and 17% experienced a Grade 4 event. Severe neutropenia was balanced in the study arms and it is a

known toxicity of docetaxel. Febrile neutropenia was experienced by 8% of those receiving the combination treatment compared to 4% of patients treated with docetaxel only

Events of Special Interest

Events that may be a class effect of Hsp90 inhibition include diarrhea, ocular toxicities, and elevations in liver enzymes.

Diarrhea is the most significant and frequently reported AE associated with the use of ganetespib. In integrated data from Studies 9090-07 and 9090-08, 48% of patients treated with both ganetespib and docetaxel experienced at least 1 AE of diarrhea compared to 15% of patients treated with docetaxel alone. In single-agent studies, approximately 78% of ganetespib patients experienced this event. The postulated mechanism of action is inhibition of EGFR in cells that line the GI tract, leading to a transient secretory diarrhea, limited to 24 to 48 hours following ganetespib infusion. This AE is manageable with loperamide or Lomotil® (atropine diphenoxylate). Prophylactic use of loperamide can reduce the occurrence of diarrhea from >80% to approximately 40%.

Ocular toxicity, manifested as visual disturbances, has been reported for several Hsp90 inhibitors. Of the 601 patients treated with ganetespib (single-agent and combination-treatment studies) as of 20 September 2013, 6 (1%) patients experienced an event of blurred vision and 4 (<1%) experienced an event of visual impairment that was assessed as related. In patients treated with single-agent ganetespib (N=378), 4 patients (1%) experienced treatment-related blurred vision and 3 patients (<1%) experienced treatment-related visual impairment. In patients treated with ganetespib in combination with docetaxel in Studies 9090-07 and 9090-08, a treatment-related visual disturbance of blurred vision was experienced by 2 (<1%) patients and a treatment-related visual disturbance of visual impairment was experienced by 1 patient. There were no such treatment-emergent events in the patients treated with docetaxel alone.

In studies using single-agent ganetespib, visual disturbances regardless of relationship to treatment included: blurred vision (5%), visual impairment (2%), and eye pain, vitreous floaters, cataract, conjunctival hemorrhage, conjunctivitis, dry eye, eyelid edema, periorbital edema, visual acuity reduced, chromatopsia, conjunctival hyperemia, eye swelling, eyelid ptosis, glaucoma, night blindness, ocular hyperemia, photopsia, and scotoma (all <1%).
In studies using ganetespib in combination with docetaxel, visual disturbances regardless of relationship to treatment included; lacrimation increased, dry eye, vision blurred (all 2%) and conjunctivitis, abnormal sensation in eye, blepharospasm, and cataract (all <1%).
In patients treated with 17-DMAG or AUY922 who experience visual disturbances, the mechanism of visual disturbances is linked to induction of apoptosis in cells in the outer nuclear layer of the retina [Zhou, J Clin Oncol, 2012]. In contrast, ganetespib did not elicit induction of apoptosis in preclinical studies using rodent models, consistent with the very low number of reported visual disturbance cases in the clinic.

Hepatocellular injuries are usually detected by enzyme elevations in serum aminotransferases (ATs), total bilirubin, and alkaline phosphatase. In the combination treatment arm of Studies 9090-07 and 9090-08, AEs of elevated aspartate aminotransferase (AST) and alanine

aminotransferase (ALT) were reported in 6% and 4%, of patients, respectively. An AE of elevated alkaline phosphatase was reported in 2% of patients and an AE of elevated bilirubin was reported in <1% of patients. Grade 3 elevations of AST and ALT were each reported in 1% of patients. In the pooled data from 378 patients who received single-agent ganetespib, AEs of elevated AST and ALT were reported in 15% and 14% of patients, respectively. An AE of elevated alkaline phosphatase was reported in 16% of these patients and an AE of elevated bilirubin was reported in 6%. Grade ≥3 elevations of AST and alkaline phosphatase were reported in 5% of patients and Grade ≥3 elevations of ALT and bilirubin in 3% of patients. None of the patients experienced concomitant elevations of ATs ≥3× the upper limit of normal (ULN) and bilirubin ≥2× ULN.

Liver toxicity in the 1st-generation geldanamycin-derivative Hsp90 inhibitors is an off-target effect. According to a study by Cysyk, the presence of benzoquinone moiety in the molecule is the suspected cause of liver toxicity [Cysyk, Chem Res Toxicol, 2006]. Ganetespib does not contain the benzoquinone moiety and, therefore, liver toxicity is not expected. This correlates with the safety information collected to date.

ECG Findings

A thorough QT study examining ECG intervals and morphology was conducted in accordance with ICH E14. The study was conducted as a sequence-randomized three-period three-way complete crossover study in healthy male volunteers. Each subject was assigned to one of six sequence groups. Each subject received 200 mg/m2 ganetespib IV over 1 hour, 400 mg moxifloxacin PO concomitantly with placebo vehicle IV over 1 hour (positive control), and placebo vehicle IV over 1 hour (negative control). Regimens were separated by 7 days. ECGs were recorded continuously over 25 hours starting 1 hour prior to infusion (active or placebo). ECGs were extracted in up to 10 replicates from each timepoint at the following times: 45, 30, and 15 minutes pre-dose, and at 0.5, 1 (immediately before infusion termination), 1.5, 2, 3, 4, 6, 8, and 24 hours post-infusion initiation. In all periods, loperamide 2 mg was administered prophylactically starting approximately 1 hour prior to drug or placebo administration, and then every 4 hours for 12 hours. The study was conducted in healthy male volunteers. Forty-five subjects received ganetespib. The dose of 200 mg/m2 was selected as this approximates the MTD, is the monotherapy dose being studied, and exceeds the combination therapy dose being studied by 33% in many studies. Intervals assessed as placebo-corrected change from baseline are denoted as "ΔΔ*interval name*," whereas those that are pre dose baseline corrected but not placebo corrected are denoted as "Δ*interval name*."

A mild heart rate increase was observed. A maximum change in heart rate of +9 bpm was observed at 6 hours post dose that returned to baseline (± 2 bpm) at 12 and 24 hours. A ΔΔQTcF (QT interval corrected by Fridericia's equation) shortening of -14 msec was observed at 6 to 8 hours post dose. At 24 hours post dose, the maximum ΔΔQTcF of 21.5 msec, with a 90% confidence interval upper bound of 23.5 msec, was observed. ΔΔQTcF returned to baseline 7 days after ganetespib dose, which was the next measurement timepoint, immediately prior to the placebo or positive control dose in the crossover. One subject exhibited QTcF > 450 msec and none exhibited greater than 480 msec after ganetespib administration. Two subjects exhibited ΔQTcF >30 msec and none exhibited ΔQTcF greater than 60 msec after ganetespib

administration. A mild prolongation in the PR interval was observed. ΔΔPR was prolonged by 11 msec at 8 and 12 hours and by 6.7 msec at 24 hours. Ganetespib did not have an effect on the QRS interval, with ΔΔQRS smaller than 0.65 msec at all post-dosing timepoints. No T-wave morphology changes were seen in subjects due to ganetespib.

These results were reviewed by an independent cardiologist who provided the following assessment:
The maximum mean ΔΔQTcF of 21.5 ms at 24 h with ganetespib places this compound in a zone of clinical ambiguity; it is not clear that this finding confers a substantial increased risk of *torsades de pointes* in patients who are being treated with ganetespib for cancer. The late occurrence of the increase in QTcF (at 24 h) is, however, unexpected. The modest increases in ΔΔHR and ΔΔPR are not likely to have clinical importance. In the clinical development program there have been 362 patients exposed to ganetespib as a single agent and 218 exposed to ganetespib plus docetaxel (total 580 patients). Only 7 subjects had prolonged QT reported as an AE, and none had *torsades de pointes* recorded on any ECG. Eight potential deaths resulting from cardiovascular SAEs have been reported and were described by investigators as cardiac arrest (n=3), sudden cardiac death (n=2) sudden death (n=1), cardiopulmonary failure (n=1) and cardiovascular insufficiency (n=1). A total of 4 of these deaths were reported among 191 subjects who received ganetespib plus docetaxel in study 9090-08. In this same study, the death of 5 of 192 subjects receiving docetaxel alone in the comparator arm was described by investigators as the result of cardiovascular SAEs. The incidence of deaths on ganetespib due to cardiovascular SAEs does not seem excessive. Risk can be minimized by appropriate patient selection, attention to electrolyte balance and ECG recording in early cycles to identify the unusual patient who may have particular sensitivity to drugs that prolong cardiac repolarization.

The results from a thorough QT study conducted in healthy volunteers (Study 9090-13) reported a modest increase in QT interval 24 hours post ganetespib dose. The mean ΔΔQTcF reached 21.5 msec. ΔΔQTcF was back to baseline 7 days after ganetespib dose. One (2%) subject exhibited QTcF > 450 msec and none greater than 480 msec after ganetespib administration. Two (4%) subjects exhibited ΔΔQTcF > 30 msec and none greater than 60 msec after ganetespib administration. To date, clinical experience with ganetespib does not support an evidence of a clinical safety risk for QTc interval prolongation and Torsade de Pointes or other uncontrolled arrhythmias. However, until the effect of ganetespib on QT interval is completely characterized, all study protocols with ganetespib single agent or combination treatment must implement updated inclusion /exclusion criteria for the cardiovascular system. ECG monitoring has also been incorporated.

Infusion Reactions

Ganetespib contains a surfactant (polysorbate 80) that has been associated with hypersensitivity reactions in other medications administered by infusion. Symptoms have included pruritus, flushing, shortness of breath, chest tightness, dizziness, headache, increased systolic BP and HR. Therefore, premedication is required prior to administration of ganetespib.

Updated guidelines for prophylaxis and management of infusion reactions have been incorporated into the protocol, based on IB Edition 9.

Preliminary Clinical Activity

Preliminary signals of clinical activity have been observed in all of the ongoing Phase 1 and Phase 1/2 clinical studies, at different dose levels and schedules.
Two subjects have achieved a Partial Response (PR) as assessed by RECIST criteria. One subject with metastatic melanoma achieved a PR after the fourth cycle of therapy and received a total of 12 cycles of treatment (48 weeks). One subject with rectal cancer achieved a PR after the second cycle of therapy and progressed after cycle 8 (total of 32 weeks of treatment). This subject had a local recurrence which was surgically removed and the subject continued on treatment at a higher dose of ganetespib.
Minor responses have also been observed. One subject with bronchoalveolar carcinoma, refractory to three previous lines of systemic therapy, had a 26% reduction in the size of the target lesions after cycle 4 and has completed a total of 13 cycles of treatment with ganetespib. One GIST subject with a known PDGFRA mutation, refractory to all standard available treatments, had an 18% reduction in tumor burden after 2 cycles and completed 8 cycles on study. Several subjects treated at higher doses (≥150 mg/m2) once weekly achieved disease stabilization for at least 4 cycles (16 weeks) of treatment.

In the phase I trial exploring weekly dosing in patients with solid tumors, 16/42 patients achieved stable disease for more than sixteen weeks and follow up is ongoing.

For the ongoing Phase 2 studies, there has been one subject (EGFR/Kras wild-type) on the 9090-06 trial who has achieved a confirmed PR as assessed by RECIST criteria, and several subjects have achieved shrinkage of baseline target and non-target lesions for ≥ 4 cycles of treatment.

Results of a randomized phase 2 study of docetaxel with or without ganetespib in patients with NSCLC who have progressed on first-line therapy have recently been presented in abstract form (Ramalingam et al, ASCO 2013). The study enrolled 252 patients with stage IIIB/IV NSCLC. The combination was well-tolerated. Mild to moderate diarrhea was observed but was manageable with antidiarrheal medications. . Grade ¾ adverse events included neutropenia, fatigue, anemia, and febrile neutropenia, which occurred with similar incidence in both arms. Patients who received the combination experienced improved overall survival (medians 7.4 versus 9.8 months). The difference was particularly pronounced in the subset of patients who were more than 6 months from initial diagnosis (medians 6.4 versus 10.7 months, $p<0.01$).

Rationale for Initial Dose

The maximum tolerated dose in the once weekly phase 1 study in solid tumor subjects (protocol 9090-02) has been determined at 216 mg/m^2. The dose to be used in this study is 200 mg/m^2. This dose level has been selected based on the expectation that there will be no pharmacological differences between 200 and 216 mg/m^2. In addition, a 200 mg/m^2 dose is within the predicted efficacious dose range based on pre-clinical models when adjusted for the human equivalent dose and would facilitate an accurate drug preparation for infusion.

2.2 Fulvestrant

Fulvestrant is a pure anti-estrogen which is thought to act by downregulating ER expression. In the United States, fulvestrant is approved for the treatment of hormone receptor-positive, metastatic breast cancer in postmenopausal women with disease progression after antiestrogen therapy.[2]

In two phase III randomized trials conducted in patients with hormone receptor-positive, metastatic breast cancer, who had progressed on either adjuvant or metastatic endocrine therapy, fulvestrant was associated with an objective response rate of 17-19%, not significantly different in comparison to the aromatase inhibitor anastrozole, where the response rate was 15-17%.[3] Median time to progression (TTP) was 5.5 months. In the EFECT trial, which enrolled patients who had previously progressed on a nonsteroidal aromatase inhibitor, fulvestrant appeared equally active compared to exemestane, and was associated with a median TTP of 3.7 months, and objective response rate of 7%.[4]. Similar results were observed in the fulvestrant control arm of CALGB 40302, with a median TTP of 4.0 months.[5]

More recently, the CONFIRM trial evaluated a different dose and schedule of fulvestrant, given as a 500 mg IM injection on days 1 and 15 of Cycle 1, followed by 500 mg IM injections on day 1 of each subsequent cycle.[6] Compared to the 250 mg once monthly schedule, there was a 1 month improvement in median PFS, leading to U.S. FDA approval of the 500 mg dose with loading schedule.

In postmenopausal women with ER-positive tumors, aromatase inhibitors (AI) are now routinely used as part of adjuvant systemic therapy. Based upon data in several phase III trials (conducted prior to the widespread use of aromatase inhibitors in the adjuvant setting), aromatase inhibitors are also widely used in the first-line metastatic setting. After progression on a nonsteroidal AI, a number of strategies are typically used in clinical practice, including a steroidal AI, fulvestrant, estradiol, and tamoxifen (Chia et al, J Clin Oncol 2008; Ellis et al, J Am Med Assoc 2009). There are no prospective trials comparing endocrine strategies in patients after AI failure, and the optimal sequencing of endocrine agents in the metastatic setting is not known. However, the 2010 National Comprehensive Cancer Center guidelines suggest that AIs, fulvestrant, tamoxifen, megestrol acetate, fluoxymesterone, and ethinyl estradiol are all reasonable options as subsequent endocrine therapy after progression on first-line endocrine therapy.

2.3 Study Disease

With over 200,000 new cases per year, breast cancer accounts for nearly one-third of all new cancer diagnoses among women in the United States.[7] Approximately 40,000 women will die of breast cancer in the U.S. alone.[7] Worldwide, breast cancer is a major cause of cancer mortality, and was responsible for approximately 458,000 deaths in the year 2008.[8]

There is increasing recognition that breast cancer is comprised of several subtypes, each with distinct features.[9] The majority of invasive breast cancers express the estrogen receptor (ER) and/or progesterone receptor (PR), and are HER2-negative. Patients with ER and/or PR-positive/HER2-negative (hereafter, designated as ER-positive) tumors tend to have a somewhat

better prognosis than patients with other tumor subtypes. However, in the metastatic setting, median survival is approximately 3 years and long-term remissions are relatively uncommon.

Initial treatment of ER-positive, metastatic breast cancer typically consists of endocrine therapy. Standard endocrine therapy options include tamoxifen, aromatase inhibitors, ovarian suppression, and fulvestrant. The choice of agent(s) varies depending on prior therapy, patient age and menopausal status, and anticipated tolerability. Although endocrine therapies can often be very effective, resistance almost invariably develops. While some patients may be salvaged with further lines of endocrine therapy, virtually all patients eventually progress through the available endocrine agents and require treatment with cytotoxic chemotherapy. For these reasons, in the metastatic setting, strategies to overcome or delay the emergence of endocrine resistance, particularly with treatments that are associated with a favorable toxicity profile in relation to standard cytotoxic agents, would be of great clinical value. Successful strategies could then be evaluated in the adjuvant setting. On average, among women with early-stage, ER-positive/HER2-negative tumors, the relative and absolute benefits of endocrine therapy far exceed that of chemotherapy.[10] Thus, efforts to improve responsiveness to endocrine agents could ultimately have a substantial impact on overall breast cancer outcomes in patients with all stages of breast cancer.

2.4 Rationale for Combining Endocrine Therapy with Ganetespib

Estrogen Receptor expression is typically conserved in metastatic lesions, compared to matched primaries.[11] Furthermore, patients who have progressed on a single line of endocrine therapy may subsequently respond to additional lines of endocrine therapy.[4,12] Thus, ER likely continues to drive a subset of metastatic breast cancers, even after progression. Hormonal therapies for ER-positive breast cancer comprise the earliest and perhaps most efficacious "molecularly targeted" approach to cancer therapy to date. Approaches to augment the efficacy of endocrine therapy and to delay the emergence of resistance have great potential to improve disease outcomes for the most common subtype of breast cancer.

While it is clear that a functional ER contributes to the growth of both hormone-sensitive and refractory tumors, further hormonal manipulation using a range of ligand-dependent therapies only manages to delay tumor progression in the vast majority of cases. Ligand-independent approaches to disrupt ER function may offer greater potential for long-term disease control.

Steroid hormone receptors such as the ER require the molecular chaperone HSP90 to achieve a mature conformation that is capable of high-affinity ligand binding and subsequent activation. These interactions are ATP-dependent, and classical HSP90 inhibitors disrupt formation of mature ER-HSP complexes by blocking ATP binding to the ATP/ADP binding pocket of HSP90. In the presence of the prototypical HSP90 inhibitor geldanamycin (GA), the ER accumulates in an intermediate complex containing a cohort of HSP that target the ER protein for degradation via the ubiquitin-proteasome pathway. As a result, receptor half-life is shortened and cellular levels profoundly decreased.

In addition, Hsp90 inhibitors deplete cellular levels of many signaling proteins, including EGFR, HER2, IGF-1R, VEGF, RAF, and AKT, which have been implicated in the development of

endocrine resistance. A number of efforts to combine endocrine therapy with a molecularly targeted agent have yielded disappointing results. For example, despite promising phase II data, the phase III study of letrozole with or without the mTOR inhibitor CCI-779 was closed early due to lack of efficacy. More recently, CALGB 40302, a randomized trial of fulvestrant with or without the dual EGFR/HER2 inhibitor lapatinib was also closed early due to lack of efficacy.[5] We hypothesize that this may be the result of selective pressure for escape pathways beyond the specific pathway targeted. The ability of HSP90 inhibitors to simultaneously deplete multiple proteins and destabilize the entire network of signaling pathways that enable endocrine resistance could overcome endocrine resistance and/or delay the emergence of endocrine resistance. Indeed, the first HSP90 inhibitor to be developed clinically, 17-AAG, has already shown promise in patients with HER2-positive breast cancer that have become resistant to trastuzumab.[13]

In preclinical models, 17-AAG markedly disrupts ER transcriptional activity in both tamoxifen-sensitive and tamoxifen-resistant MCF-7 cells.[14] Tumor progression in mice bearing xenografts established using MCF-7 cells that demonstrate tamoxifen dependence for growth in vivo was significantly inhibited by 17-AAG administered on a standard dose and schedule. Further work has demonstrated that geldanamycin, at concentrations with no single agent anti-tumor activity, can virtually eradicate the outgrowth of tamoxifen-resistant MCF-7 cells in culture.

In comparison to 17-AAG, ganetespib is a potent, second-generation, small-molecule HSP90 inhibitor with a chemical structure unrelated to the ansamycin family of first-generation HSP90 inhibitors (e.g. 17-AAG or IPI-504). In preclinical studies, STA-9090 has shown potency up to 100 times greater than the first generation HSP90 inhibitors as well as potency across a wider range of kinases. STA-9090 has activity against a wider range of cancer types. Work by Whitesell et al (personal communication) has demonstrated more potent anti-proliferative activity against MCF-7 breast cancer cells than 17-AAG (IC50 ~25 nM versus 250-500 nM). Furthermore, it was more effective at limiting the emergence of tamoxifen-resistant clones and in cultures of MCF-7 cells and was synergistic with tamoxifen in a xenograft model.[15] More recently, preliminary evidence of synergistic activity of fulvestrant and STA-9090 has been demonstrated in cell lines, and xenograft studies are underway.

2.5 Summary

This is a randomized phase II trial evaluating fulvestrant with or without ganetespib, a potent, novel HSP90 inhibitor. The primary clinical objective is progression-free survival. We hypothesize that ganetespib will augment the efficacy of fulvestrant and be associated with an improvement in progression-free survival.
Secondary clinical endpoints include objective response rate (among patients with measurable disease at baseline), safety/tolerability, clinical benefit rate (CR + PR + SD >/= 6 months), and overall survival.
The correlative objectives of this study include evaluation of the relationship between HSF1 and clinical outcome, alterations in heat-shock network function, ER expression and transcriptional activity, and alterations in other relevant downstream targets. Patients who enroll on this trial will also be approached regarding an ^{18}FES-PET companion study (which will be written as an entirely separate, IRB-approved protocol) when it is available.

3. PARTICIPANT SELECTION

Laboratory tests required for eligibility must be completed within 14 days prior to randomization. Baseline measurements must be documented from tests within 28 days prior to randomization. QTc must be documented by 12-lead ECG within 28 days prior to randomization. Other non-laboratory tests must be performed with the timeframe specified.

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Participants must have histologically confirmed invasive breast cancer that is metastatic or unresectable locally advanced. Histological documentation of metastatic/recurrent breast cancer is not required if there is unequivocal evidence for recurrence of the breast cancer.

3.1.2 Estrogen and/or progesterone receptor positive breast cancer, as determined by pathology from either primary or metastatic site(s). Central confirmation is not required.

3.1.3 HER2 negative, defined as 0-1+ by immunohistochemistry or FISH-negative (ratio < 2.2). Central confirmation is not required.

3.1.4 Postmenopausal women are eligible. Postmenopausal is defined as any of the following:

- Age > 60 years
- Age > 45 with intact uterus and amenorrhea for 12 months or more
- Follicle stimulating hormone (FSH) levels within postmenopausal range according the ranges established by the testing facility
- Premenopausal women who have been on a GnRH agonist for at least 3 consecutive months prior to study entry are eligible. Women in this group MUST remain on the GnRH agonist for the duration of protocol treatment.
- Status-post bilateral oophorectomy-After adequate healing post surgery.

3.1.5 Women and men, age $\geq$ 18 years of age

3.1.6 Measurable disease is required. Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$ 20 mm with conventional techniques or as $\geq$ 10 mm with spiral CT scan. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable. See section 10 for details. **As of 4/30/14, the evaluable/non-measurable cohort has been filled and only patients with measurable disease are allowed moving forward. Prior to 4/30/14, up to 20% of patients entered on this trial (i.e. 14 patients) were entered with evaluable but nonmeasurable disease.**

3.1.7 Endocrine resistant breast cancer, defined as either:

- Relapsed while on adjuvant endocrine therapy or within 1 year of completion of adjuvant endocrine therapy

-OR-

- Progression through at least one line of endocrine therapy for metastatic or locally advanced breast cancer. There is no limit on the number of prior endocrine therapies received.

3.1.8 Patients may have received up to one prior line of chemotherapy for metastatic or unresectable locally advanced breast cancer.

3.1.9 Patients may have initiated bisphosphonate therapy prior to start of protocol therapy. Bisphosphonate therapy may continue during protocol treatment. Such patients will have bone lesions considered evaluable for progression.

3.1.10 Patients must be at least 2 weeks from prior chemotherapy or radiotherapy, or any investigational drug product, with adequate recovery of toxicity to baseline, or grade $\leq$1, with the exception of alopecia and hot flashes. There is no washout period for prior endocrine therapy.

3.1.11 ECOG Performance Status 0-1 (Appendix A)

3.1.12 Availability of a tissue block from initial breast cancer diagnosis and/or metastatic recurrence. If a tissue block is not available, 10-20 unstained slides may be provided as an alternative. If unstained slides will be provided, they should not be sent until specifically requested by the DFCI study coordinator.

3.1.13 For patients with biopsy-accessible disease, patients must be willing to undergo a required on-treatment research biopsy. This biopsy will occur on ~Cycle 2 Day 9.

3.1.14 Biopsies may be done with local anesthesia or intravenous conscious sedation, according to standard institutional guidelines.

3.1.15 Research biopsies requiring general anesthesia are not allowed on this protocol unless a biopsy is being obtained simultaneously for clinical reasons, in the judgment of the patients' treating physician.

3.1.16 Patients who undergo an attempted on-treatment research biopsy and in whom inadequate tissue is obtained are still eligible to continue protocol therapy. They will not be required to undergo a repeat biopsy attempt.

3.1.17 Participants must have normal organ and marrow function as defined below:

- Absolute neutrophil count $\geq$ 1,500/mcL
- Platelets $\geq$ 100,000/mcL
- Hgb $\geq$ 9 mg/dL(which may be post transfusion)
- Total bilirubin < 1.5 X institutional upper limit of normal (patients with documented Gilbert's disease are allowed total bilirubin up to 3X ULN)
- AST (SGOT)/ALT (SGPT) $\leq$ 2.5 X institutional upper limit of if no liver metastases; and < 5 X institutional upper limit if liver metastases are present
- Creatinine $\leq$ 2X institutional upper limit of normal

3.1.18 Adequate IV access

3.1.19 Fulvestrant is contraindicated in pregnancy (category D). The effects of ganetespib on the developing human fetus are unknown. For these reasons, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Premenopausal women who have been on a GnRH agonist for at least 3 consecutive months prior to study entry are eligible. Women in this group MUST remain on the GnRH agonist for the duration of protocol treatment. Such patients should be counseled that GnRH agonists alone may not be adequate contraception and that adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation should be employed.

3.1.20 Ability to understand and the willingness to sign a written informed consent document.

3.1.21 Patients on therapeutic dose anti-coagulation with warfarin, LMWH, or other anti-coagulants will be allowed to participate in the study. However, the anti-coagulants should be held pre- and post- research biopsy (when applicable) as per institutional standards. The risks of a temporary hold of anti-coagulation should be carefully considered and explained to the patient as part of the informed consent process. If it is not felt to be in the best interest of the patient to have anti-coagulation held, the patient may still enter the study, but should not undergo a research biopsy.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Prior treatment with an HSP90 inhibitor (e.g. 17-AAG, IPI-504, AUY922, STA-9090 (ganetespib) etc.)

3.2.2 Prior treatment with fulvestrant.

3.2.3 Participants may not be receiving any other investigational agents.

3.2.4 Concurrent treatment with commercial agents or other agents with the intent to treat the participant's malignancy, including endocrine therapy, chemotherapy, and/or targeted therapy, with the exception of bisphosphonates and GnRH agonists, as detailed in Section 3.1.8 and 3.1.10.

3.2.5 Untreated or progressive brain metastases. Patients with treated brain metastases not requiring chronic corticosteroids for symptom control are eligible.

3.2.6 Pending visceral crisis, in the opinion of the treating investigator.

3.2.7 History of allergic reactions attributed to compounds of similar chemical or biologic composition to fulvestrant or ganetespib.

3.2.8 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.9 Serious cardiac illness, defined as follows:

- Clinically unstable cardiac disease, including unstable atrial fibrillation, symptomatic bradycardia, unstable congestive heart failure, active myocardial ischemia, or indwelling temporary pacemaker
- Ventricular tachycardia or a supraventricular tachycardia that requires treatment with a Class Ia antiarrhythmic drug (e.g., quinidine, procainamide, disopyramide) or Class III antiarrhythmic drug (e.g. sotalol, amiodarone, dofetilide). Use of other antiarrhythmic drugs is permissible.
- Use of medications that have been linked to the occurrence of torsades de pointes (see Appendix I for a list of such medications). Investigators should note that Zofran (ondansetron) has been linked to QTc prolongation and the occurrence of torsades de pointes. Therefore it should not be used in patients being treated with ganetespib. The use of all other serotonin 5 HT3 antagonists is acceptable (e.g., palonsetron, granisetron, tropisetron).
- Second-or third degree atrioventricular block (AV block) unless treated with a permanent pacemaker.
- Complete left bundle branch block (LBBB)
- History of long QT syndrome or a family member with this condition

3.2.10 Corrected QTc>470msec. The corrected QTc may be corrected using either Bazett's or Friderica's formula. In general QTcf is the preferred correction method.

3.2.11 Pregnant women are excluded from this study because fulvestrant is contraindicated in pregnancy (category D) and ganetespib is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with fulvestrant or ganetespib, breastfeeding should be discontinued if the mother is treated on either arm of the trial.

3.2.12 Individuals with the following cancers are eligible if diagnosed and definitively treated prior to study entry: cervical cancer *in situ*, basal cell or squamous cell carcinoma of the skin. Individuals with a history of other malignancy are ineligible unless disease-free for at least 3 years or deemed by the investigator to be at low risk (<10%) for recurrence of that malignancy.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Both men and women are eligible for this protocol. Every effort will be made to include patients from minority populations. Because breast cancer predominantly affects females, it is anticipated that male enrollment will be <5% of the overall study population.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol treatment. 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 may be cancelled. Notify the QACT Registrar of registration cancellations as soon as possible.

4.2 Registration Process for DF/HCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the QACT protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical and/or research chart. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies. (Note—there are currently no active ancillary studies associated with this trial.)

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

4. The QACT Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant when applicable.

5. An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration and/or randomization.

4.3 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the Participating Institution and faxed or e-mailed to the Coordinating Center at CTOPM@dfci.harvard.edu or by fax at 617-632-5152:

- Signed informed consent form
- HIPAA authorization form (if separate from the informed consent document)
- Completed QACT Eligibility Checklist
- Copy of required laboratory tests including: Hematology (CBC w/differential), Serum Chemistries (potassium, BUN, creatinine, magnesium, total bilirubin, SGOT (AST), SGPT (ALT), and Alkaline Phosphatase, tumor markers (CEA and CA27-29), and pregnancy test (for women of child-bearing potential only).
- EKG report
- Confirmation of archival tissue availability (primary and/or metastatic sites)
- Radiologic imaging report including bone scan
- Pathology report and documentation of ER/PR and HER2 status

- Clinic visit note documenting history and physical exam

The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:

- Register the participant on the study with the DF/HCC QACT
- Upon receiving confirmation of registration by the QACT, the Coordinating Center will inform the Participating Institution and provide the study specific participant case number, and if applicable the dose treatment level.

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

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

5. TREATMENT PLAN

Treatment must begin within 7 days of randomization. Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for fulvestrant and ganetespib are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Patients who enter the study and who are postmenopausal on the basis of GnRH agonist treatment must continue that treatment throughout the duration of the study.

A total of 71 patients will be enrolled in the study. Patients will be stratified according to whether or not they have measurable disease (by RECIST 1.1) at baseline and then randomized 1:2 to the two treatment arms:

Arm A: Fulvestrant (n=24)
Arm B: Fulvestrant plus Ganetespib (n=47)

Patients who are randomized to Arm A may cross over to Arm C (Fulvestrant plus ganetespib) at the time of centrally confirmed progression.

In both Arms A and B, each cycle will be ~28 days in length. Treatment will continue until disease progression (possibly the second progression on the combination therapy, if the patient was originally randomized to Arm A fulvestrant), unacceptable toxicity, withdrawal of consent, or death, whichever comes first. Follow up (specifically, tumor measurements as scheduled in

the protocol) will continue until disease progression, withdrawal of consent, death, or the start of other anti-neoplastic therapy, whichever comes first.

Minor schedule changes owing to observed holidays, inclement weather, etc. are permitted. Patients may interrupt therapy for protocol-directed reasons (i.e. toxicity). From Cycle 3 forward, patients may also interrupt therapy for personal preferences (e.g., holidays, vacations). Treatment should resume according to protocol guidelines. Patients may not voluntarily omit fulvestrant at any time. From Cycle 3 forward, patients may not voluntarily omit more than 2 doses of ganetespib in a single cycle, except as detailed below. See Section 9.0 Study Calendar for additional details.

It is anticipated that some patients in Arm B or Arm C may either choose or be taken off of ganetespib for reasons other than disease progression (i.e., for toxicity or patient preference). The following guidelines apply:

- Patients who are taken off of ganetespib for toxicity at any time should remain on study and continue fulvestrant. Restaging evaluations and clinic visits should continue on the protocol mandated schedule until either disease progression, intercurrent illness that prevents further administration of treatment, unacceptable adverse event(s), the participant decides to withdraw from the study, or general or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

- Patients being treated with fulvestrant + ganetespib whose disease remains stable or in response for at least 6 cycles may elect to discontinue ganetespib. Once ganetespib is discontinued, it will not be restarted. Patients who discontinue ganetespib should remain on study and continue fulvestrant. Restaging evaluations and clinic visits should continue on the protocol mandated schedule until either disease progression, intercurrent illness that prevents further administration of treatment, unacceptable adverse event(s), the participant decides to withdraw from the study, or general or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

As part of the clinical trial, ganetespib will be provided free of charge to study participants by Synta Pharmaceuticals. Patients and/or their insurance companies will be billed for the cost of fulvestrant, which will come from commercial supply.

Arm A					
Agent	**Pre-medications**	**Dose**	**Route**	**Schedule**	**Cycle Length**

Fulvestrant	None	500 mg	Intramuscular injection	C1: D1, D15 C2: D1 Then on Day 1 of each subsequent cycle	28 days (4 weeks)

Arm B: Dose Level 1					
Agent	**Pre-medications***	**Dose**	**Route**	**Schedule**	**Cycle Length**
Fulvestrant	None	500 mg	Intramuscular injection	C1: D1, D15 C2: D1 Then on Day 1 of each subsequent cycle	28 days (4 weeks)
Ganetespib	• Suggest 2 mg Loperimide 1-2 hrs before infusion. • Dexamethasone 10 mg IV, or therapeutic equivalents • Diphenhydramine HCL 25-50 mg, or therapeutic equivalents *(dose of diphenhydramine may be reduced per investigator discretion if patient had previous problems with 25mg or higher)*	200 mg/m^2	Intravenous (see section 5.2.1 for vascular access device restrictions)	D 1,8,15	

***See section 5.2 for management of diarrhea and hypersensitivity reactions. For patients who have successfully tolerated at least 2 cycles of ganetespib without a hypersensitivity reaction, routine premedication with dexamethasone and diphenhydramine prior to subsequent ganetespib infusions is not required and is left to the discretion of the treating provider.*

At the time of initial protocol design, it was decided that if more than 2 DLTs were observed within the first 10 patients randomized to Arm B, study accrual would be temporarily suspended, pending review by the Study PI and the Synta medical monitor (Section 14.2). If the DLTs were confirmed, the study would re-open at Dose Level -1 (i.e., 175 mg/m2); if the DLTs were not confirmed (i.e. the events did not meet the definition of DLT), the study would re-open at Dose Level 1 (i.e., 200 mg/m^2). This DLT rule was not triggered for the first 10 patients randomized to Arm B and enrollment has continued at Arm B Dose Level 1.

Arm B: Dose Level -1					
Agent	**Pre-medications***	**Dose**	**Route**	**Schedule**	**Cycle Length**
Fulvestrant	None	500 mg	Intramuscular injection	C1: D1, D15 C2: D1 Then on Day 1 of each subsequent cycle	28 days (4 weeks)
Ganetespib	• Suggest 2 mg Loperimide 1-2 hrs before infusion. • Dexamethasone 10 mg IV, or therapeutic equivalents • Diphenhydramine HCL 25-50 mg, or therapeutic equivalents *(dose of diphenhydramine may be reduced per investigator discretion if patient had previous problems with 25mg or higher)*	175 mg/m^2	Intravenous (see section 5.2.1 for vascular access device restrictions)	D 1,8,15	

****** See section 5.2 for management of diarrhea and hypersensitivity reactions. For patients who have successfully tolerated at least 2 cycles of ganetespib without a hypersensitivity reaction, routine premedication with dexamethasone and diphenhydramine prior to subsequent ganetespib infusions is not required and is left to the discretion of the treating provider.*

Patients who are randomized to Arm A may cross over to Arm C (Fulvestrant plus ganetespib) at the time of centrally confirmed progression. Patients in Arm C will start at Dose Level 1, as shown below. At the time of initial protocol design, it was decided that if the Arm B starting dose was dropped to Dose Level -1, as detailed above, then the Arm C starting dose would also be dropped to Dose Level -1. Since the DLT rule was not triggered for the first 10 patients randomized to Arm B, the Arm C starting dose has continued at Arm B Dose Level 1.

Arm C: Dose Level 1					
Agent	**Pre-medications***	**Dose**	**Route**	**Schedule**	**Cycle Length**
Fulvestrant	None	500 mg	Intramuscular injection	C1: D1, C2: D1 Then on Day 1 of each subsequent cycle	28 days (4 weeks)
Ganetespib	• Suggest 2 mg Loperimide 1-2 hrs before infusion. • Dexamethasone 10 mg IV, or therapeutic equivalents • Diphenhydramine HCL 25-50 mg, or therapeutic equivalents *(dose of diphenhydramine may be reduced per investigator discretion if patient had previous problems with 25mg or higher)*	200 mg/m^2	Intravenous (see section 5.2.1 for vascular access device restrictions)	D 1,8,15	

***** *See section 5.2 for management of diarrhea and hypersensitivity reactions. For patients who have successfully tolerated at least 2 cycles of ganetespib without a hypersensitivity reaction, routine premedication with dexamethasone and diphenhydramine prior to subsequent ganetespib infusions is not required and is left to the discretion of the treating provider.*

Arm C: Dose Level -1					
Agent	**Pre-medications***	**Dose**	**Route**	**Schedule**	**Cycle Length**
Fulvestrant	None	500 mg	Intramuscular injection	C1: D1, \ C2: D1 Then on Day 1 of each subsequent cycle	28 days (4 weeks)
Ganetespib	• Suggest 2 mg Loperimide 1-2 hrs before infusion. • Dexamethasone 10 mg IV, or therapeutic equivalents • Diphenhydramine HCL 25-50 mg, or therapeutic equivalents *(dose of diphenhydramine may be reduced per investigator discretion if patient had previous problems with 25mg or higher)*	175 mg/m^2	Intravenous (see section 5.2.1 for vascular access device restrictions)	D 1,8,15	

***** See section 5.2 for management of diarrhea and hypersensitivity reactions. For patients who have successfully tolerated at least 2 cycles of ganetespib without a hypersensitivity reaction, routine premedication with dexamethasone and diphenhydramine prior to subsequent ganetespib infusions is not required and is left to the discretion of the treating provider.*

5.1 Study Procedures

At the time of registration, the eligibility checklist and supporting documentation to verify eligibility must be provided prior to randomization. Data will be collected and maintained on study specific case report forms. See Section 9.0 Study Calendar for additional details.

5.1.1 Screening

All subjects must sign an informed consent document prior to initiation of any study related procedures. The informed consent document must be signed within 30 days of randomization. Baseline laboratory evaluations must be completed within 14 days of randomization. Baseline measurements including scans must be completed within 28 days of randomization. QTc must be documented by 12-lead ECG within 28 days of randomization. The remaining screening procedures must be conducted within 28 days of randomization.

- Review of study eligibility criteria
- Medical history
- Breast cancer history
- Record concomitant medications
- ECOG performance status
- Laboratory assessments
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
    - Serum HCG, in women of childbearing potential
- 12-lead ECG
- Review of baseline symptoms
- Radiologic imaging studies to evaluate tumor status. Contrast computed tomography (CT) (or without contrast if contraindicated) of chest and abdomen is preferred. Magnetic resonance imaging (MRI) of chest and abdomen is an acceptable alternative. Note: PET-CT scans are *not allowed* per protocol for primary assessment of response. PET-CT scans may be done according to clinical judgment to supplement CT or MRI scans in cases where results of the PET-CT may clarify or resolve a clinical question.
- Measurements of palpable/visible lesions, if used as a target lesion (for example, chest wall nodule)
- Bone scan
- Request and collect blocks from initial breast cancer diagnosis and/or recurrent/metastatic lesion(s). See section 9.5. If unstained slides will be provided as an alternative, they should not be cut or sent until specifically requested by the DFCI Study Coordinator.
- Consider optional baseline research biopsy for patients with easily accessible disease. See section 9.5.

5.1.2 Cycle 1, Day 1

- Clinic visit

- Record concomitant medications
- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical examination
- Height and weight
- ECOG performance status
- Record baseline symptoms (using CTCAE grading criteria)
- Laboratory assessments
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
    - CA 27-29, CEA
- 12-lead ECG
- Research blood draw for whole blood, PBMC, Streck tube, and CTC collection. See Section 9.5.
- Fulvestrant administration via intramuscular injection
- Ganetespib i.v. administration via silicone VADs (Arm B only)

5.1.3 Cycle 1, Day 2 (Arms B and C only)

- 12-lead ECG required 24 hours after Ganetespib infusion. See section 5.6.

5.1.4 Cycle 1, Day 8 (Arm B only)

- Clinic visit
- Record concomitant medications
- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical examination
- Weight
- ECOG performance status
- Record AEs (using CTCAE v4 grading criteria)
- Laboratory assessments
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
- Ganetespib administration via silicone VADS

5.1.5 Cycle 1, Day 15

- Clinic visit
- Record concomitant medications

- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical examination
- Weight (Arm B only)
- ECOG performance status
- Record AEs (using CTCAE v4grading criteria)
- Laboratory assessments (Arm B only)
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
- Fulvestrant administration via intramuscular injection
- Ganetespib administration via silicone VADS (Arm B only)

5.1.6 Cycle 2, Day 1

- Clinic visit
- Record concomitant medications
- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical examination
- Weight (Arm B only)
- ECOG performance status
- Record AEs (using CTCAE v4.0 grading criteria)
- Laboratory assessments
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
- 12-lead ECG (Arms B and C only). If pre-dose QTc >470 ms a repeat ECG is required approximately 24 hours after the Ganetespib dose.
- Fulvestrant administration via intramuscular injection
- Ganetespib administration via silicone only VADS (Arm B only)

5.1.7 Cycle 2, Day 8

- Research blood draw for PBMC and CTC analysis. See Section 9.5 and Appendix C for details.

For patients randomized to Arm B, <u>Cycle 2 Day 8 draw research blood between 2-4 hours after the Day 8 dose of ganetespib</u>. If the Day 8 dose is held for any reason (e.g., for toxicity), draw research blood between 2-4 hours after the Day 15 dose of <u>ganetespib</u>.

5.1.8 Cycle 2, Day 9

- Research blood draws for PBMC and CTC analysis. See Section 9.5 and Appendix C for details.
- Tissue biopsy for patients with biopsy-accessible disease. See Section 9.5 and Appendices B and C for details.

For patients randomized to Arm A:, Cycle 2 Day 9 biopsy may be performed +/- 3 days and on the same day as the Cycle 2 Day 9 research blood draw (1st research blood draw to occur on Cycle 2 Day 8 +/- 3 days). Draw the Cycle 2 Day 9 research blood samples 18-48 hours after the Cycle 2 Day 8 research blood draw. Patients on Arm A who do not undergo the Cycle 2 Day 9 research biopsy are not required to undergo the Cycle 2 day 9 research blood draw.

For patients randomized to Arm B, collect Cycle 2 Day 9 biopsy sample and research blood draw between 18-48 hours after the Cycle 2 Day 8 dose of ganetespib. *If the Day 8 dose is held for any reason (e.g., for toxicity), collect the biopsy sample and research blood draw between 18-48 hours after the Cycle 2 Day 15 dose of ganetespib.*

5.1.9 Cycle 2, Days 8 and 15 (Arm B only)

- Vital signs (heart rate, blood pressure, and respiratory rate)
- Weight
- Laboratory assessments
  - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
  - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
  - Serum calcium, magnesium, phosphorus
- Ganetespib administration via silicone VADS

5.1.10 Cycle 3 Day 1, and Day 1 of Subsequent Cycles

- Clinic visit
- Record concomitant medications
- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical examination
- Weight (Arm B only)
- ECOG performance status
- Record AEs (using CTCAE v4 grading criteria)
- Laboratory assessments
  - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count

  - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
  - Serum calcium, magnesium, phosphorus
  - CEA, CA27-29, if elevated at baseline. In patients with elevated markers at baseline, tumor markers should subsequently be drawn once every 2 cycles.
- 12-lead ECG (Arms B and C only). If pre-dose QTc >470 ms a repeat ECG is required approximately 24 hours after the Ganetespib dose. See section 5.6 for additional ECG requirements for patient at moderate to high risk of torsades de pointes.
- Radiologic imaging studies to evaluate tumor status. Contrast computed tomography (CT) (or without contrast if contraindicated) of chest and abdomen is preferred. Magnetic resonance imaging (MRI) of chest and abdomen is an acceptable alternative. Note: PET-CT scans are *not allowed* per protocol for primary assessment of response. PET-CT scans may be done according to clinical judgment to supplement CT or MRI scans in cases where results of the PET-CT may clarify or resolve a clinical question.
- Measurements of palpable/visible lesions, if used as a target lesion (for example, chest wall nodule)
- Follow-up imaging and measurements should be done every 2 cycles (8 weeks) and every 2-4 cycles (8-16 weeks) for patients who have been on study for >1 year. Imaging can be done +/- 1 week from targeted date but before start of the subsequent cycle.

5.1.11 Days 8 and 15 of Cycle 3 and Subsequent Cycles (Arm B only)

- Vital signs (heart rate, blood pressure, and respiratory rate)
- Weight
- Laboratory assessments
  - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
  - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
  - Serum calcium, magnesium, phosphorus
- Ganetespib administration via silicone VADS

5.1.12 Time of Crossover from Arm A to Arm C (applies only to patients initially randomized to Arm A)

- Clinic visit
- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical exam
- Weight
- ECOG performance status
- Record AEs (using CTCAE v4.0 grading criteria)
- Record concomitant medications

- Laboratory assessments
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
    - CEA, CA 27-29
- 12-lead ECG
- Research blood draw for CTCs, Streck tube, PBMCs. See section 9.5.
- Optional research biopsy. See section 9.5.
- Bone scan
- Measurements of palpable/visible lesions, if used as a target lesion (for example, chest wall nodule)
- Review of films by central radiology reviewer (DF/HCC Tumor Imaging Metrics Core) and confirmation of progression by overall study PI (REQUIRED before patient receives first dose of ganetespib)

5.1.13 Prior to Removal from Protocol Treatment (applies to Arms A, B, and C)

- Review of films by central radiology reviewer (DF/HCC Tumor Imaging Metrics Core) and confirmation of progression by overall study PI is REQUIRED for patient to be taken off protocol treatment in either arm, if off-treatment reason is progression.
- "Real-time" central review for progression is not required if the off-study reason is other than progression (i.e. for toxicity, etc.); however, central review will be performed at a later time in order to adjudicate the primary endpoint (PFS)

5.1.14 End of Protocol Treatment (within 30 days after the last dose of protocol treatment)

- Clinic visit
- Vital signs (heart rate, blood pressure, and respiratory rate)
- Physical exam
- Weight
- ECOG performance status
- Record AEs (using CTCAE v4.0 grading criteria)
- Laboratory assessments
    - Hematology: hemoglobin, hematocrit, white blood cell count with differential, platelet count
    - Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin
    - Serum calcium, magnesium, phosphorus
- Research blood draw for CTCs, PBMCs, and Streck tube. See section 9.5.
- Optional research biopsy. See section 9.5.

5.1.15 PFS Follow-up Procedures

Because the primary endpoint of the study is PFS, patients who are taken off treatment for any reason other than disease progression will REMAIN ON PROTOCOL and continue with the protocol mandated schedule of events (i.e. staging studies) until progressive disease, start of new anti-cancer therapy, or death, whichever comes first. For example, patients who are randomized to Arm B (fulvestrant + ganetespib) and who discontinue ganetespib without evidence of progression should continue on fulvestrant alone and continued to be followed for progression with staging studies/evaluations per protocol.

5.1.16 Survival Follow-up Procedures

Subject survival information will be collected every 3 months (+/- 2 weeks) from date of last dose of study drug (first treatment they we assigned to) until the subject's death, or until the subject is lost to follow-up, or up to 2 years from last dose of study drug, whichever comes first.
For patients who receive post-protocol care at a participating institution, survival information will be collected from medical records, supplemented by direct contact with a patient's treating physician. Patients who receive post-protocol care outside of a participating institution will be directly contacted by telephone. If a patient or patient representative is not able to be successfully contacted, then the patient's local physician will be contacted as an alternative. Prior to deeming a patient lost to follow-up, a search will also be made of the Social Security Death Index and/or the National Death Index. At the time of final survival reporting, any patients with missing survival data will also be searched through the Social Security Death Index and/or the National Death Index. The consent form will include information detailing the follow up procedures in this trial. The following information will be collected:

- The subject's survival status, and if deceased, the date of death.
- The method by which the survival status was assessed and the date it was assessed.
- The cause of death, if known
- Categorization of cause of death, if known (e.g. disease progression, protocol-related toxicity, other treatment-related toxicity, other, or unknown).

5.2 Agent Administration

5.2.1 Ganetespib

- Administration -- 200 mg/m^2 IV D1, 8, 15 of a 28-day cycle (i.e. 3 weeks on, 1 week off)
- Ganetespib should be infused over 1 hour using non-PVC, non-DEHP IV bags and tubing with a 0.2µm in-line filter.
- Based on preclinical data, use of vascular access devices (VADs) (such as ports and peripherally-inserted central catheters [PICCS]) containing silicone catheters or peripheral IV is permitted. ***Use of VADs with catheters made of any other material***

is not permitted. Following ganetespib administration through a VAD, care should be taken to flush the line after each dose of study drug.

- For patients with a pre-existing port: Prior to the first ganetespib administration, the treating investigator must send the name of the port device to the overall study PI and Coordinating Center contact and the overall study PI and/or her designate must approve the device prior to use for ganetespib administration.
- For patients who will have a port placed during the study: prior to port placement, the treating investigator must send the name of the intended port device to the overall study PI and Coordinating Center contact and the overall study PI and/or her designate must approve the device prior to the insertion procedure.

- Premedications:
  - Dexamethasone 10 mg IV, or therapeutic equivalents (**Required**)
  - Diphenhydramine HCL 25-50 mg, or therapeutic equivalents (**Required**)
    - *Dose of diphenhydramine may be reduced per investigator discretion if patient experienced previous problems with 25mg or higher*
  - Loperamide (Imodium®): Start dosing (2mg) 1-2 hour**s before** ganetespib infusion (Suggested)
  - *For patients who have successfully tolerated at least 2 cycles of ganetespib without a hypersensitivity reaction, routine premedication with dexamethasone and diphenhydramine prior to subsequent ganetespib infusions is not required and is left to the discretion of the treating provider.*
- No pre- or post-hydration is required
- Dose modifications are outlined in Section 6

5.2.2 Fulvestrant

- Administration – 250 mg/5mL (x2) for a total of 500 mg via intramuscular injection on Cycle 1 Day 1, Cycle 1 Day 15, and then on Day 1 of each subsequent cycle.
- No dose modifications.
- Commercial supply of fulvestrant will be used.

5.3 Definition of Dose-Limiting Toxicity

There are no phase I data with the combination of fulvestrant + ganetespib. Based upon the observed toxicities to date, mechanism of action, and metabolisms, no additive toxicities are anticipated. However, for the first 10 patients assigned to Arm B, we will include an early stopping rule for Cycle 1 dose-limiting toxicity (DLT). The DLT assessment period will be from Cycle 1 Day 1 until Cycle 2 Day 1.

Dose-limiting toxicity (DLT) is based on the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE v4.0). DLT refers to toxicities experienced during the first cycle of treatment. A DLT will be defined as follows:

- Grade 3 or 4 hematologic or non-hematologic toxicity thought likely or definitely related to protocol therapy will be counted as a DLT, with the exception of:
    - Grade 3 diarrhea that responds to maximal supportive measures. If grade 3 diarrhea is persistent > 7 days despite maximal supportive measures, it will be counted as a DLT
    - Grade 3 fatigue
    - Grade 3 hot flashes
    - Grade 3 electrolyte disturbance, unless persistent for > 7 days despite maximal supportive measures.

Management and dose modifications associated with the above adverse events are outlined in Sections 5.4 (General Concomitant Medication and Supportive Care Guidelines) and Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications).

5.4 General Concomitant Medication and Supportive Care Guidelines

Medications taken prior to Cycle 1, Day 1 and during the study will be collected and noted in the medical record. The following are the supportive care guidelines for the study. The guidelines apply to all Arms of the study, except as otherwise noted:

- For patients receiving ganetespib, it is preferred not to use concomitant use of medications that are associated with a high incidence of QT prolongation (e.g., quinolones and ondansetron). See Appendix I for a list of medications that are known to predispose to Torsades de Pointes.
- No other antineoplastic agents will be permitted during this study, with the exception of bisphosphonates and GnRH agonists under the guidelines as specified in Section 3.1.8 and 3.1.10.
- No concurrent radiation treatment will be permitted during this study
- No treatment with chronic immunosuppressants (e.g., cyclosporine following transplantation or systemic steroids for treatment of autoimmune disease) will be permitted during this study; however, use of inhalant steroids and steroids given for antiemetic purposes are permitted
- For patients receiving ganetespib, caution should be exercised when concomitant medications that are cleared predominantly by the CYP3A4 or CYP2C19 pathways are administered. Examples of compound classes metabolized predominantly by CYP3A4 or CYP2C19 are provided below:
    - CYP3A4 substrates: benzodiazepines (e.g. midazolam), anticonvulsants (e.g. carbamazepine), calcium channel blockers (e.g. felodipine, nifedipine), antineoplastics (e.g. vincristine), macrolide antibiotics (e.g. erythromycin) and HIV antiviral agents.
    - CYP2C19 substrates: proton pump inhibitors (e.g. omeprazole) and antidepressants (e.g. sertraline).

The above information is precautionary only. Investigators are encouraged to discuss the use of individual medications with the Principal Investigator on a case-by-case basis.

5.5 Supportive Care Guidelines

Patients should receive full supportive care, including antiemetics, blood transfusions, intravenous fluid, etc., as clinically appropriate. The reason(s) for treatment, dosage, and dates of treatment should be recorded in the medical record.

Antiemetics

Patients may be given antiemetics at the discretion of the treating physician.

Anti-infectives

Antibiotics may be used at the discretion of the treating physician. If there is a reasonable alternative, it is preferred that quinolones and other agents that may prolong QTc are avoided.

Bisphosphonates

Oral or intravenous bisphosphonate treatment is allowed if the first dose was given prior to initiation of protocol therapy. Patients already receiving bisphosphonate therapy at the time of study entry may continue the treatment. Patients with new bone metastases documented as part of study screening procedures may begin a bisphosphonate, provided the first dose is given prior to initiation of protocol-based therapy. Bisphosphonates may not be started after the initiation of protocol-based therapy.

Growth factors

Patients may receive erythropoietin or other specific red blood cell growth factors and red blood cell transfusions will be permitted as clinically indicated during the study.
Patients may receive bone marrow colony stimulating factors (such as granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor) as clinically indicated during the study.

If an Investigator determines a patient is at risk for severe neutropenia or febrile neutropenia, granulocyte-colony stimulating factor (G-CSF) may be used prophylactically beginning with the first cycle. G-CSF prophylactic use is recommended during subsequent treatment cycles in case of neutropenia lasting more than 7 days, febrile neutropenia, or documented infection with neutropenia.

Diarrhea management guidelines

Many patients will experience diarrhea, and some patients may experience Grade 3 or 4 diarrhea. The following proactive and ongoing management principles are necessary to avoid more serious complications of diarrhea. However, guidelines such as these should never replace sound clinical judgment.

Experience suggests that diarrhea is an expected drug class effect for Hsp90 inhibitors and it typically starts 2-3 hours following administration of ganetespib in most patients. However,

when appropriately managed with anti-diarrhea treatment, it is generally mild to moderate and its duration limited to 24 hours.

Due to the risk of diarrhea while receiving ganetespib, all patients will be educated to use prophylactic medication (Lomotil® or Imodium®) before ganetespib administration and before the onset of gastrointestinal symptoms to mitigate the severity and duration.

Suggested prophylaxis regimens are as follows:

- Loperamide (Imodium®): Start dosing (2mg) 1-2 hours **before** ganetespib infusion and continue with 2 mg every four hours for 12 hours. This treatment may be continued for up to 24 hours in the absence of symptoms of diarrhea. Due to inter-patient variability, adjustment to this regimen should be made on a case-by-case basis. The maximum dosage of loperamide is 16 mg/24 hrs.
- Dietary counseling: foods/liquids and quantities to intake or avoid

Guidelines for Treatment of Diarrhea (Active Management):

- In the event of diarrhea, patients will take loperamide at an initial 4 mg dose followed by 2 mg doses every 4 hours. In the presence of uncomplicated grade 1 or 2 diarrhea, loperamide will be continued until the patient is free from diarrhea for 12 hours. Total daily dose may not exceed 16 mg (8 capsules)
- If mild to moderate diarrhea persists after 24 hours despite treatment with loperamide, a cocktail of atropine-diphenoxylate (lomotil) or equivalent and loperamide may be considered. Loperamide 2 mg may be alternated with one tablet of Lomotil every 3 hours.
- For grade 3 or 4 diarrhea or complicated grade 1 or 2 (severe cramping, severe nausea/vomiting, decreased PS, fever, sepsis, grade 3 or 4 neutropenia, frank bleeding, dehydration), IV fluids will be used as appropriate, as well as prophylactic antibiotics. Hospitalization is recommended.
- Grade 1-2 : Loperamide 4mg first dose, then 2 mg every 4 hours until free from diarrhea for 12 hours
- If Grade 1 or 2 diarrhea persists for more than 24 hours: Loperamide 2mg q 2 hrs
- If Grade 1 or 2 diarrhea persists after 48 hours: Start 2L agents: a cocktail of Dyphenoxylate and atropine sulfate (Lomotil®). Alternate 2 mg loperamide with one tablet of Lomotil every 3 hours.
- Grade 3 or 4 or Complicated Grade 1 or 2: IV fluids as appropriate.

<u>Management of hypersensitivity reactions</u>

Ganetespib contains a surfactant (polysorbate 80) that has been associated with hypersensitivity reactions in other medications administered by infusion. Infusion type reactions associated with ganetespib (approximately 7% of patients) have been reported at doses of 10 to 200 mg/m^2. Symptoms included pruritus, flushing, shortness of breath, chest tightness, dizziness, headache, increased systolic blood pressure and heart rate. These events were mostly grade 1 and grade 2 and usually responsive to treatment with i.v. diphenhydramine HCl (Benadryl®) and steroids

(sometimes with H2 blockers). In addition, subjects with infusion-type reactions usually could continue receiving ganetespib after pre-mediation with diphenhydramine HCl and steroids.

Based on IB Edition 9, premedication is required prior to ganetespib infusion for at least 2 cycles. Premedications include:

- Dexamethasone 10 mg IV, or therapeutic equivalents
- Diphenhydramine HCL 25-50 mg, or therapeutic equivalents
    - *Dose of diphenhydramine may be reduced per investigator discretion if patient experienced previous problems with 25mg or higher*

For patients who have successfully tolerated at least 2 cycles of ganetespib without a hypersensitivity reaction, routine premedication with dexamethasone and diphenhydramine prior to subsequent ganetespib infusions is not required and is left to the discretion of the treating provider.

If, despite premedication, an infusion hypersensitivity reaction to ganetespib is suspected, the following management is provided as guidance only; treatment will be based on clinical presentation. Institution specific premedication and/or treatment procedures and regimens may also be appropriate in lieu of these guidelines.

- **Mild and moderate symptoms:**

    1. Stop ganetespib administration
    2. Give i.v. diphenhydramine HCl (Benadryl®) 25-50 mg and dexamethasone 10 mg i.v.
    3. After recovery from symptoms, resume ganetespib infusion or re-schedule patient for re-treatment; a reduced flow rate may be considered

In subsequent cycles, consider optimizing premedication regimen.

- **Severe symptoms** (i.e., hypotension requiring pressor therapy, IV fluids, angioedema, respiratory distress requiring bronchodilator therapy, generalized urticaria):

    1. Stop ganetespib administration
    2. Give intravenous dexamethasone and diphenhydramine HCl (Benadryl®) as above
    3. Add intravenous H2 blocker
    4. Add adrenaline (1:1000) or bronchodilators as indicated
    5. Reschedule dose administration for the following day
    6. In subsequent cycles, consider optimizing premedication regimen.

If severe symptoms reoccur with optimal premedication, treatment with ganetespib should be discontinued permanently.

The following is an example of an optimized premedication regimen:

- Dexamethasone 12 mg PO and diphenhydramine HCL 25-50 mg PO approximately 12-24 hours prior to the next dose of ganetespib
- Repeat dexamethasone 12 mg PO and diphenhydramine HCL 25-50 mg PO approximately 4-6 hours prior to the re-challenge

5.6 Cardiovascular Guidelines

In response to findings of a modest increase in QT interval 24 hours post-dose, additional ECG monitoring is being incorporated into the protocol pending data from an ECG evaluation that is being performed within the context of Study 9090-14.

5.6.1 Patients at High to Moderate risk for torsades de pointes

For patients deemed at baseline by the treating investigator to be at moderate to high risk for torsades de pointes, the overall study PI should be consulted prior to registration to confirm the patient's eligibility for the study, given that patients with significant cardiovascular co-morbidities are excluded from the trial. It is not anticipated that patients with moderate or high risk of torsades will generally be enrolled onto the study. In the rare instance that such a patient meets all eligibility requirements, including cardiovascular eligibility requirements, ECGs are required at the following timepoints for patients receiving ganetespib:

- Baseline
- Cycle 1 Day 2 (approximately 24 hours post Cycle 1 Day 1 ganetespib dose)
- Pre-dose on Cycle 2 Day 1
- Approximately 24 hours post Cycle 2 Day 1 dose
- Pre-dose for each subsequent cycle
- Approximately 24 hours post Day 1 of each subsequent cycle

5.6.2 Patients at Low risk for torsades de pointes

For patients deemed at baseline by the treating investigator at low risk for torsades de pointes, ECG monitoring *in triplicate* is required at the following time points for patients receiving ganetespib:

- Baseline
- Cycle 1 Day 2 (approximately 24 hours post Cycle 1 Day 1 ganetespib dose)
- Pre-dose on Cycle 2 Day 1

If there is no evidence of QTc prolongation (QTc > 470 msec), subsequent EKGs are required:

- Pre-dose, day 1 of each cycle

If there is evidence of QTc prolongation (QTc > 470 msec) on the pre-dose EKG, the patient is required to return for a repeat EKG ~24 hours post-ganetespib dose (applies to all cycles of treatment).

If a patient has a reported QTc ≥ 501 ms (QTc prolongation Grade 3 severity), at any ECG (an average of triplicate recordings), the patient may continue ganetespib treatment at a reduced dose of 120 mg/m2 after the QTc has decreased to at least < 470 ms.

If a patient has a reported QTc ≥ 501 ms or > 60 ms change from baseline and torsades de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia (QTc prolongation Grade 3 severity) or repeated Grade 3 or higher QTc prolongation, the patient must discontinue treatment with ganetespib.

If a patient has a dose reduction or discontinues treatment due to QTc prolongation, the patient should have additional ECG monitoring in triplicate until the QTc interval returns to baseline.

Patients with QTc prolongation of grade 3 severity or higher should be closely monitored during the first 72 hours post ganetespib infusion (i.e. electrolytes, concomitant medications) and at subsequent cycles of ganetespib treatment.

Because of the QT study findings of a modest increase in QT interval prolongation at 24 hours post dose, certain drugs should not be used by patients being treated with ganetespib. The use of any medication that has the potential for QTc prolongation **and** has been linked to the occurrence of torsades de pointes is strictly prohibited. See Appendix I for a list of drugs with a risk of torsades de pointes. Investigators should note that Zofran (ondansetron) has been linked to QTc prolongation and the occurrence of torsades de pointes. Therefore it should not be used in patients being treated with ganetespib. The use of all other serotonin 5 HT3 antagonists is acceptable (e.g., palonsetron, granisetron, tropisetron).

Medications that have the potential of prolonging the QT interval but are not linked to the occurrence of torsades de pointes should be avoided or used with caution. The decision to use such a medication should be made by the Investigator, taking into consideration the patient's medical history and current QTc value. In addition, Investigators should optimize patients' electrolyte status and correct serum magnesium, potassium, and calcium as clinically indicated.

5.7 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),

- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.
- At the discretion of the MD, investigator, or Sponsor

Patients who are randomized to Arm A may cross over to Arm C and receive combination therapy with fulvestrant + ganetespib at the time of centrally confirmed progression. Treatment may then continue on the combination until one of the above criteria applies.

It is anticipated that some patients in Arm B or Arm C may either choose or be taken off of ganetespib for reasons other than disease progression (i.e. for toxicity or patient preference). The following guidelines apply:

- Patients who are taken off of ganetespib for toxicity at any time should remain on study and continue fulvestrant. Restaging evaluations and clinic visits should continue on the protocol mandated schedule until either disease progression, intercurrent illness that prevents further administration of treatment, unacceptable adverse event(s), the participant decides to withdraw from the study, or general or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

- Patients being treated with fulvestrant + ganetespib whose disease remains stable or in response for at least 6 cycles may elect to discontinue ganetespib. Once ganetespib is discontinued, it will not be restarted. Patients who discontinue ganetespib should remain on study and continue fulvestrant. Restaging evaluations and clinic visits should continue on the protocol mandated schedule until either disease progression, intercurrent illness that prevents further administration of treatment, unacceptable adverse event(s), the participant decides to withdraw from the study, or general or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

- Patients who are taken off fulvestrant and/or ganetespib for any reason other than centrally-confirmed progression or death should be followed according to study calendar D until evidence of disease progression, start of new anti-cancer therapy, withdrawal of consent for follow-up, or death, whichever comes first.

5.8 Duration of Survival and Adverse Event Follow Up

Participants will be followed for survival for up to 4 years after the last dose of protocol therapy or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.9 Criteria for Removal from Study

Participants will be removed from study treatment when any of the following occur:

- Disease progression,
- Start of new anti-cancer therapy, or
- Participant decides to withdraw from the study treatment
- Subject death

Note: Patients in Arm A who progress on fulvestrant alone may first cross over to Arm C (fulvestrant + ganetespib).
Participants will be considered 'Off Study' when one of the following events occurs:

- Death
- Participant decides to withdraw from the study follow-up procedures
- 4 years after the date of the last study treatment dose

Note: Patients on either arm who discontinue assigned protocol treatment (fulvestrant and/or ganetespib) for any reason other than progression or death *should not be removed from study* but instead should continue to be followed per protocol (See Study Calendar D) until evidence of disease progression, start of new anti-cancer therapy, withdrawal of consent for follow-up, or death, whichever occurs first.

The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator (or Protocol Chair), Dr. Nancy Lin at 617-632-3352, page # 42012.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) v 4.0 which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Adverse Event Lists(s) for ganetespib

Aggregate clinical data across all dose levels from the ongoing clinical studies show the following adverse events (AEs) of all grades as most common (>10%) by the Medical Dictionary for Regulatory Activities (MedDRA) preferred term:

- Diarrhea (72%)
- Abdominal pain (22%)
- Fatigue (51%)
- Insomnia (16%)
- Dyspnoea (17%)
- Anorexia (22%)
- Increase in aspartate aminotransferase (AST) (14%)
- Increase in alkaline phosphatase (AP) (13%)
- Nausea (37%)
- Vomiting (20%)
- Peripheral edema (15%)
- Anemia (24%)
- Constipation (15%)
- Increase in alanine aminotransferase (ALT) (11%)
- Headache (18%)
- Back pain (15%)
- Dizziness (13%)
- Hypokalemia (11%)
- Pyrexia (11%)
- Dehydration (10%)
- Skin rash (10%)

Diarrhea is experienced by virtually all subjects treated at therapeutic doses. Based on clinical experience to date, over-the-counter medications such as dyphenoxylate and atropine sulfate (Lomotil®) and loperamide (Imodium®) mitigate the severity and duration of the diarrhea. It is recommended that medications, such as those listed above, should be utilized before ganetespib administration and before the onset of GI symptoms. (See Protocol section 5.5 for recommended dosing instructions)

Fatigue is observed in up to 50% of patients, mostly NCI CTCAE grade 1. Some subjects have experienced grade 2 or 3 fatigue, which was managed by study drug dose reduction

per protocol. In some subjects, medications (e.g. Ritalin) were used successfully to manage fatigue.

To date, severe liver toxicity has not been observed. In some subjects, transient elevations of transaminases or bilirubin have been observed, typically CTCAE grade 1 or 2; however a few grade 3 elevations were observed.

6.1.2 Adverse Event List(s) for fulvestrant

The side effect profile includes injection site pain (9-12% of patients), nausea (10-13%), and bone pain (~10%). In the CONFIRM trial, the most commonly reported toxicities (any grade) on the 500 mg arm were GI disturbances (20%), joint disorders (19%), injection site reactions (14%), and hot flashes (8%).[6] The most common grade 3 or higher toxicities were GI disturbances (2%) and joint disorders (2%). Other grade 3 or higher toxicities were uncommon and did not exceed 1% frequency in this study. Please refer to the package insert for a comprehensive list of adverse events.

6.2 Dose Modifications/Delays

Dose delays and modifications will be made using the following rules. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) v 4.0 which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.2.1 Ganetespib

Dose Level	Dose	Route	Schedule	Cycle Length
Starting dose	200 mg/m^2	IV	D1, 8, 15	28 days
First dose reduction	175 mg/m^2	IV	D1, 8, 15	28 days
Second dose reduction	150 mg/m^2	IV	D1, 8, 15	28 days

As detailed in Sections 5.1 and 14.2, at the time of initial study design, it was decided that in the case of more than 2 DLTs within the first 10 patients on Dose Level 1 (starting dose 200 mg/m^2), the study would close temporarily to accrual while the events were adjudicated. Based upon results of this adjudication, the study would re-open at Dose Level -1 (starting dose 175 mg/m^2). In this event, there would be only 1 allowed dose reduction (i.e. to 150 mg/m^2) for patients entered at this starting dose level. Once decreased, doses would NOT be re-escalated. This DLT rule was not triggered for the first 10 patients randomized to Arm B; therefore enrollment to Arm B has continued at Arm B Dose Level 1 and treatment on Arm C has continued at Arm B Dose Level 1.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

The following guidelines are provided for the modification of dose of ganetespib in the event of toxicities attributed by the investigator to this drug. Toxicities unrelated to ganetespib will not ordinarily result in modification of ganetespib dose.

Once a dose has been reduced, it should not be re-escalated.

Missed doses should be *skipped*, rather than held. Therefore, one cycle will nearly always be 4 weeks, allowing time for vacation, holidays, etc.

Dose Modification Guidelines for Ganetespib

Toxicity	Grade	Guideline for management	Ganetespib dose modification*
Diarrhea	1	See Section 5.5	None
	2	See Section 5.5	None; If unacceptable to patient or medically concerning despite maximal supportive measures, then skip until recovery to ≤ grade 1 (or baseline), up to 21 days*. Restart at same dose**.
	≥ 3		If grade 3 or greater despite maximal supportive measures, skip until recovery to ≤ grade 1 (or baseline), up to 21 days*. And then Reduce 1 dose level.
Nausea/vomiting	1 or 2	Treatment as appropriate.	None
	3 (First or 2nd occurrence)	Treatment as appropriate.	Skip until recovery to ≤ grade 1 (or baseline), up to 21 days*. And then Restart at same dose.
	3 (3rd or more occurrence)		If grade 3 or higher despite optimal prophylactic measures, then skip until recovery to ≤ grade 1 (or baseline), up to 21 days*. And then Reduce 1 dose level.
Fatigue	1	No intervention	None
	2	Treatment as appropriate.	None; If unacceptable to patient or medically concerning then hold until recovery to ≤ grade 1 (or baseline), up to 21 days*. Restart at same dose**.
	3	Treatment as appropriate.	Skip until recovery to ≤ grade 1 (or baseline), up to 21 days*. And then Reduce 1 dose level.
Other non-Hematologic toxicity	1	No Intervention	None
	2	Treatment as appropriate	None; If unacceptable to patient or medically concerning then skip until recovery to ≤ grade 1 (or baseline), up to 21 days*. Restart at same dose **.
	2 prolonged or clinically significant and grade ≥ 3	Treatment as appropriate	Skip until recovery to ≤ grade 1 (or baseline), up to 21 days And then Reduce 1 dose level*

Toxicity	Grade	Guideline for management	Ganetespib dose modification*
Infusion reactions	3	Treatment per institutional guidelines for infusion reactions	Contact Principal Investigator to determine whether patient may be retreated
	4	Stop infusion	Permanently discontinue
Thrombocytopenia	1 or 2	No intervention	None
	3	No intervention	Skip until recovery to ≤ grade 1 (or baseline), up to 21 days And then Reduce 1 dose level*
Hemoglobin	1 or 2	Treatment as appropriate	None
	3	Treatment as appropriate	Skip until recovery to ≤ grade 1 (or baseline), up to 21 days And then Reduce 1 dose level*
Neutropenia	1 or 2	No intervention	None
	3 (ANC < 1,000/μL) lasting > 7 days		Skip until recovery to ≤ grade 1 (or baseline), up to 21 days And then Reduce 1 dose level*
Cardiac toxicity	3 (**first** occurrence of QTc ≥ 501 ms on average of triplicate recordings)		Reduce dose level to 120 mg/m^2 after the QTc has decreased to at least < 470 ms.
	3 (**repeat** occurrence of QTc ≥ 501 ms on average of triplicate recordings)		Permanently discontinue
	4 (QTc ≥ 501 ms or > 60 ms change from baseline and torsades de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia		Permanently discontinue

* if no recovery after 21 days, patients should go off study

** if dose has been previously held for grade 2 toxicity and grade 2 symptoms recur, OR if the patient finds the symptoms unacceptable, hold dose until recovery to ≤ grade 1 and then reduce dose one level

In the case of grade 4 hematologic or non-hematologic toxicity, ganetespib should be skipped until recovery to grade 1 or less (or baseline), up to 21 days, and then the dose reduced to 150 mg/m^2. Subjects unable to tolerate ganetespib at a dose level of 150 mg/m^2 or whose ganetespib related toxicity has not returned to baseline or Grade ≤ 1 within 21 days should stop ganetespib. Patients may continue on fulvestrant alone at this time. Once permanently discontinued, ganetespib should not be restarted.

In the case of a recurrent grade 3 event after prior dose reduction, ganetespib should be held until the related toxicity has returned to baseline or Grade ≤ 1. Subjects should be

reduced by 1 additional dose level (i.e. 2 dose levels from baseline) upon restarting study drug. Only 2 dose reductions are allowed per protocol.

6.2.2 Fulvestrant

Fulvestrant will be administered at a dose of 500 mg via intramuscular injection on Days 1 and 15 of Cycle 1, and then on Day 1 of each subsequent 28-day cycle.

There are no dose modifications of fulvestrant. Commercial supply fulvestrant will be administered in this study. The cost of fulvestrant will be billed to the patient and/or her insurance company.

If fulvestrant-related adverse events occur, they should be managed using standard clinical practice.

If grade 3 or 4 toxicity occurs that the treating investigator feels is related to fulvestrant, the study Principal Investigator should be contacted to discuss the case and determine whether continuation of protocol therapy is appropriate.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Ganetespib (STA-9090)

Refer to the Investigator's Brochure.

7.1.1 Description

Classification: HSP-90 inhibitor

Molecular formula: $C_{20}H_{20}N_4O_3$ **M.W**.: 364.40 g/mol

Physical description: white to off white powder

Ganetespib (STA-9090), chemical name *5-[2,4-dihydroxy-5-(1-methylethyl) phenyl]- 2,4-dihydro-4-(1-methyl-1H-indol-5-yl)-3H-1,2,4-triazole-3-one,* is a novel triazolone heterocyclic compound. Its molecular formula is $C_{20}H_{20}N_4O_3$. STA-9090 is a white to off white powder with a molecular weight of 364.40 g/mol.

7.1.2 Form

Ganetespib 300mg/vial (25mg/mL)

Each vial contains 12mL of deliverable ganetespib (12.84mL total including an overage per USP requirements) at a concentration of 25 mg/mL in a PEG 300, polysorbate 80 and dehydrated alcohol cosolvent-surfactant system. The drug product is a colorless to slightly yellow, clear solution, essentially free of visible particles. The drug product vial can be identified with a dark blue cap and applicable label.

Caution: Ensure that the ganetespib 300mg/vial (25mg/mL), is used with the instructions provided below. (Another drug product ganetespib 8 mg/mL is available that uses different instructions for preparation)

7.1.3 Storage and Stability

The Ganetespib 300mg/vial drug product should be stored at 20°C - 25°C (68°F – 77°F) with excursions allowed between 15°C and 30°C (59°F and 86°F) (USP Controlled Room Temperature). Alternatively, ganetespib may be store in a cool place between 8°C (46°F) and 20°C (68°F). DO NOT FREEZE.

7.1.4 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.

7.1.5 Availability

Ganetespib is an investigational agent and will be supplied free-of-charge by Synta Pharmaceuticals.

7.1.6 Preparation

The dilution of ganetespib (STA-9090) (25 mg/mL) will be with D5W (5% Dextrose for Injection) to a concentration range of 0.1 mg/mL-1.1 mg/mL. Deviation from the proposed concentrations must be approved by the sponsor prior to administration. Infusion of the study drug must be completed within 4 hours once diluted with D5W.

Eligible subjects will receive ganetespib under the direction of a physician identified on the form FDA 1572. STA-9090 administration will be given over 60 minutes. The amount of ganetespib administered will be determined by the dose level to which the subject is assigned at study entry and by calculating the subject's body surface area (BSA).

Preparation Instructions for ganetespib 300mg/vial (25mg/mL) Infusion Solution
Study drug preparation should be performed under standard aseptic conditions following chemotherapeutic precautions.

In general, the following concentration range guidelines should be used when preparing ganetespib for infusion. Sponsor approved deviations from these recommendations may be necessary based on an individual subject's BSA and dose level. However, at no time should a concentration be outside the range of 0.1 - 1.1mg/mL. The target infusion volume should always be 500mL.

1. Calculate the total dose of ganetespib the subject is to receive based on their BSA:
   Total ganetespib dose (mg) = ganetespib dose (mg/m^2) X BSA (m^2)

2. Calculate the total volume of ganetespib drug product Solution (25mg/mL) needed:
   Total ganetespib Solution (mL) = Total ganetespib dose (mg) from Step 1 / 25mg/mL

3. Calculate the total volume of 5% Dextrose for Injection (D5W) needed for preparation of the 500mL infusion solution:
   Total D5W Volume (mL) = 500mL – Total ganetespib Solution (mL) from Step 2

4. Note: This step is required only for the Ganetespib 300/ml vial (25mg/ml) drug product if stored at 2°C-8°C.

   Remove the appropriate number of ganetespib vials from the refrigerator and let them sit at ambient room temperature for approximately 20 minutes.

5. Into an empty 500mL, non-PVC, non-DEHP containing infusion bag or a 500mL glass bottle, transfer the calculated amount of D5W in Step 3 using a syringe and a needle. The D5W volume can also be adjusted to the calculated D5W volume in Step 3 if pre-filled bags/bottles are used.
   Caution: Non-PVC, non-DEHP bags must be used.

6. Using a syringe and a 16G or 18G needle carefully withdraw the calculated amount of the ganetespib drug product solution from Step 2 and inject into the infusion bag containing D5W.
   Note: Because of the viscosity of the drug product the 16G needle is recommended for ease of withdrawal.

7. Carefully squeeze the transfer ports, 4 corners of the bag, and the middle of the bag to ensure complete mixing of the drug product and D5W. Continue mixing by gently inverting the bag end-over-end 10 times. Visually inspect the final infusion solution. The final infusion solution should be clear and essentially free of any visible particles.
   Caution: Do not use if a clear solution is not obtained. Contact the study Sponsor.

8. Attach the non-PVC, non-DEHP containing infusions set with a 0.22µm end-filter to the infusion bag.
   Caution: Non-PVC, non-DEHP tubing with a 0.22µm filter must be used.

9. Store the infusion solution at room temperature until use avoiding direct exposure to light. Upon completing the infusion solution preparation, the one hour administration of the infusion solution must be completed within 4 hours. The time needed to prepare the dosing solution does not count against the 4 hour limit. There is no need to protect the IV bag from ambient light during the infusion.
   Caution: Do not refrigerate the infusion solution.
   Caution: The 1-hour infusion must be complete within 4 hours of completing the infusion solution preparation.

7.1.7 Administration

1. Set up the infusion pump to deliver the 500mL ganetespib infusion solution over 60 minutes

   Note - Use of Vascular Access Devices:

   Based on preclinical data, use of vascular access devices (VADs) (such as ports and peripherally-inserted central catheters [PICCS]) containing silicone catheters are permitted.

   Use of VADs with catheters made of any other material is not allowed.

   Following ganetespib administration through a VAD, care should be taken to flush the line after each dose of study drug. Please follow routine clinical practice for care of patients utilizing VADs.

2. At the end of the infusion the IV tubing **must be flushed with D5W** to ensure complete delivery of the required dose of ganetespib. The infusion rate of the D5W flush should be at the same rate as the drug infusion.
3. Dispose of used IV bags, tubing and used drug vials per institutional guidelines.

7.1.8 Ordering

Ganetespib will be ordered and supplied by Synta Pharmaceuticals once the protocol is approved. Please refer to Appendix G: Section 4.0 for additional information.

7.1.9 Accountability

The investigator, or a responsible party designated by the investigator, must 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.)

7.1.10 Destruction and Return

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

7.1.11 Pharmacokinetics

The pharmacokinetics of STA-9090 administered at various doses to subjects with solid tumors on a weekly or twice-weekly schedule is under investigation in 2 Phase I trials.

Preliminary data and calculated parameters are available in subjects with solid tumors for doses up to 259 mg/m^2. C_{max} and AUC increase in approximate proportion to dose irrespective of dosing day with virtually identical dose-exposure ratios for Day 1 and Day 15, indicating linear pharmacokinetics and a lack of drug accumulation. Distribution and elimination phases of STA-9090 show maximum concentration at the end of the infusion declining by approximately 10-fold within the first hour and nearly 100-fold within 10 hours following infusion termination. Mean terminal half-lives have ranged from approximately 5.2 to 14 hours. STA-9090 plasma concentrations on Day 1 and 15 are comparable following either once or twice weekly dosing, indicating the lack of drug accumulation.

7.1.12 Toxicity

Given that current clinical data are limited, continued careful monitoring for the following adverse events based upon STA-9090 nonclinical toxicology assessments: cytopenias including lymphopenia and neutropenia; infection; loose stools, diarrhea, or bloody diarrhea; nausea/vomiting; dehydration; electrolyte disturbances; abnormal serum chemistries (i.e. liver function tests, creatinine, CPK); injection site reactions; pulmonary symptoms; bone infarction/bone pain; and skin rash. Pronounced local reactions may occur if STA-9090 is extravasated or improperly administered. Animal toxicology studies may not predict adverse events in humans. Therefore other unanticipated adverse events may occur and careful general clinical monitoring is warranted.

Reported adverse events for other HSP90 inhibitors include, but are not limited to, nausea, vomiting, diarrhea, dehydration, stomatitis, anemia, neutropenia, thrombocytopenia, hepatotoxicity, bilirubinemia, anorexia, fatigue, myalgia, hypoxia, dyspnea, pulmonary infiltrates, cardiac toxicity, pancreatitis, fever, and hypersensitivity reactions. Given that some of these adverse events may be mechanism-based, careful monitoring for the adverse events listed above is recommended.

Gastrointestinal symptoms have been the most frequently observed AEs and include diarrhea, nausea, vomiting, abdominal pain and bloating. Due to the potential for dehydration, subjects should be advised to maintain appropriate hydration. Blood chemistries, including electrolytes, should be regularly monitored, and corrected as appropriate.

Fatigue is observed frequently, in up to 50% of subjects; mostly NCI CTC Grade 1. Anemia, typically NCI CTC Grade 1 was reported in approximately 30% of subjects, although the relationship to study drug was unclear. No other bone marrow suppressive effects were reported. To date, severe liver toxicity has not been observed and liver toxicity was not the dose-limiting toxicity in the Phase I trials. In some subjects, transient elevations of transaminases or bilirubin were observed, typically NCI CTC Grade 1 or 2, however a few Grade 3 elevations were observed.

Interim analysis of ECG intervals suggests that administration of ganetespib is associated with an acute, modest, transient decrease in heart rate (5-10 bpm) and accompanying

increase in P-R interval (NCI CTC Grade 1). These changes were not symptomatic and did not lead to brady-arrhythmias. Mean QRS did not appear to change in any consistent way. Similar observations have been described for other HSP90 inhibitors and seem to represent a drug class effect.

7.2 Fulvestrant

Fulvestrant is commercially available and considered standard-of-care for patients with hormone receptor positive, metastatic breast cancer. The dose, schedule, and mode of administration used in this trial are identical to the dose, schedule, and mode of administration approved for use for this indication by the U.S. FDA.

Fulvestrant will be charged to the patient and/or insurance company as it is considered standard-of-care.

7.2.1 Description

Fulvestrant injection is commercially available as a sterile, single patient, prefilled syringe containing 250 mg at a concentration of 50 mg/mL. The solution is a clear, colorless to yellow, viscous liquid. In addition to the fulvestrant, each injection contains as inactive ingredients alcohol, USP, benzyl alcohol, NF, and benzyl benzoate, USP, as co-solvents and castor oil, USP, as a co-solvent and release rate modifier.

7.2.2 Storage and Stability

Syringes of fulvestrant should be stored in the original container and refrigerated at 2-8°C.

7.2.3 Preparation

Remove syringe from tray and check that it is not damaged. Peel open the safety needle outer packaging. Break the seal of the white plastic cover on the syringe luer connector to remove the cover with the attached rubber tip cap. Twist to lock the needle to the luer lock connector.
Remove needle sheath. Remove excess air from the syringe (a small bubble may remain).

7.2.4 Administration

Fulvestrant will be administered at a dose of 500 mg (2 x 250 mg injections) IM on Cycle 1 Day 1, Cycle 1 Day 15, Cycle 2 Day 1, and on Day 1 of each subsequent cycle. Cycle length = 28 days.

Fulvestrant will be administered slowly into each buttock.

Immediately upon withdrawal of syringe from patient activate needle protection device by pushing lever arm forward until needle tip is fully covered.

Visually confirm that the needle arm has advanced and that the needle tip is fully covered. If unable to activate, discard immediately into an approved sharps container.

7.2.5 Toxicity

The side effect profile includes injection site pain (9-12% of patients), nausea (10-13%) and bone pain (~10%). In the CONFIRM trial, the most commonly reported toxicities (any grade) on the 500 mg arm were GI disturbances (20%), joint disorders (19%), injection site reactions (14%), and hot flashes (8%).[6] The most common grade 3 or higher toxicities were GI disturbances (2%) and joint disorders (2%). Other grade 3 or higher toxicities were uncommon and did not exceed 1% frequency in this study.

Please refer to the package insert for a comprehensive list of adverse events.

7.2.6 Drug Interactions

In *in vitro* studies of human hepatocytes, fulvestrant is metabolized predominantly by glucuronidation. The metabolites are thought to have no estrogenic activity and minimal anti-estrogenic activity.

In studies using human liver microsomes, fulvestrant inhibited the activity of CYP1A2, 2C9, and 3A4 minimally and in healthy volunteer studies fulvestrant did not affect metabolism of CYP3A4 substrates. CYP3A4 did metabolize fulvestrant in these studies; however the human hepatocyte studies noted above indicate conjugation is a more important metabolic pathway. In addition, studies in healthy volunteers indicate that fulvestrant metabolism is not significantly affected by inducers or inhibitors of CYP3A4, nor does fulvestrant affect metabolism of CYP3A4 substrates. Thus, fulvestrant is not expected to be involved in significant drug interactions mediated by CYP3A4.

As per Chouinard, et al., fulvestrant is glucuronidated by UGT1A1, 1A3, 1A4 and 1A8 and ganetespib has been shown to inhibit UGT-mediated metabolism of furosemide (UGT1As); however, laboratory work completed at Synta specifically addressing possible UGT-mediated interactions with ganetespib indicates that an alteration of fulvestrant metabolism by ganetespib is unlikely. Fulvestrant metabolism is unaffected by the presence of ganetespib at ganetespib concentrations of 1, 10, and 20 μM in human hepatocytes *in vitro*. For reference, at 216 mg/m^2 ganetespib, C_{max} is approximately 16 μM. At 150 mg/m^2 it is approximately 11 μM (9090-02).

Synta also addressed DDI potential from the perspective of a possible effect of fulvestrant on ganetespib *in vitro*. There was no alteration of ganetespib metabolism by fulvestrant. Concentrations of 0.3 and 3 μM fulvestrant and 1, 10, and 20 μM of ganetespib were used.

8. CORRELATIVE/SPECIAL STUDIES

See section 9.5 for details on specimen collection, processing, and shipping instructions.

The biomarker studies are designed to be hypothesis generating. It is anticipated that promising findings will be validated in the context of future studies.

The key determinants of whether a tumor will or will not respond to Hsp90 inhibition remain poorly defined. The basal level of HSP90 per se has not proven to be predictive. Although activation of the heat shock response in surrogate normal tissue or even tumor tissue can serve as a sensitive pharmacodynamic endpoint for Hsp90 inhibition, it also fails to predict anticancer response. The frustrating lack of predictive markers arises, to a great extent because HSP90 does not act alone, but rather as part of a complex network of additional heat shock proteins, co-chaperones and accessory molecules. This network's architecture makes the potential for cross-talk and compensation enormous. It also explains the disappointing lack of power seen for elevation of any single heat shock protein as an independent predictor of outcome in cancers including breast. We hypothesize that activation of Heat Shock Factor 1 (HSF1), the major transcriptional regulator of inducible expression for the entire heat shock network, can serve as an indicator of the relative level of proteotoxic stress within a particular tumor and consequently the "load" on the Hsp90-based chaperone machinery**.** This load could in turn determine which patients are more or less likely to derive clinical benefit from endocrine therapy alone or the combination of endocrine therapy + HSP90 inhibitor. In pilot work using commercial tissue microarrays, we have found clear immunohistochemical evidence for HSF1 activation in ~40-50% of the ER+ breast cancer cases. We have also found a correlation between HSF1 activation and patient outcomes among patients with ER+ breast cancer within the Nurses' Health Study (Santagata et al, unpublished data, manuscript in preparation).

As detailed in Section 1.4, we will collect archival tumor specimens, on-study biopsy specimens, and blood specimens at a variety of timepoints in order to explore a series of correlative aims.

As the primary correlative endpoint, HSF1 will be measured semi-quantitatively in archived breast tumor specimens (primary and/or metastatic; whatever is available for a particular patient) by immuno-histochemistry (IHC) according to methods we have developed within the Nurses' Health Study (Santagata et al, unpublished data, manuscript in preparation). The level of HSF1 mRNA and that of a panel of genes known to be regulated at the transcriptional level by HSF1 will be measured using a custom Gene Expression CodeSet and the nCounter Analysis System developed by Nanostring Technologies**.** The custom CodeSet designed for this study will include an activation signature consisting of sequences regulated by HSF1. Genes reported to be estrogen-regulated which could be impacted by fulvestrant and/or ganetespib therapy will be included as well as other breast cancer progression-related genes such as hormone receptors and kinases**.** Cytokeratins will be included in an effort to normalize for tumor/epithelial cell content of specimens being analyzed. With multiplexed target profiling of up to 800 transcripts in a single reaction, nCounter Gene Expression CodeSets deliver gold-standard levels of sensitivity, precision and linearity across a wide range of basic research and translational medicine applications. No amplification is required and protocols have been developed that permit

quantitative analysis starting with 100ng or less of total RNA from formalin-fixed tissue as well as raw cell or blood lysates**.** These features make possible the analysis of very small amounts of tissue available from archived materials and core biopsies.

As a control for tumor tissue, NanoString CodeSets will be used to analyze normal peripheral blood lymphocytes (PBL) obtained from the same patient before and after drug exposure. Levels of the highly inducible HSP70 family member HSP72 have been used as a pharmacodynamic endpoint in many previous HSP90 inhibitor trials, but measurements were made at the protein level by more cumbersome, less sensitive and less quantitative immunoassays. Although HSP72 has clearly not been predictive of anticancer response in patients, no patient data are available on the drug-induced modulation of other heat shock protein classes or the wide range other genes included in our custom CodeSet. Likewise, no data are available on whether fulvestrant and its modulation of ER signaling will alter regulation of the heat shock response in either normal or tumor tissue.

As a complement to monitoring RNA expression signatures in biopsy specimens by NanoString CodeSet, ER, PR, AR, and HSF1 will be assessed at the protein level in circulating tumor cells (CTCs) before and after drug treatment. CTCs offer a non-invasive approach to obtain and characterize metastatic breast cancer cells, but their usefulness has been limited by the very low yields available from conventional isolation methods. Krop et al have recently reported an improved technique that provides a much higher yield of relatively pure CTCs, facilitating their molecular characterization. Typically starting from 7.5 ml of whole blood, a median tumor cell number of 117 can be obtained. The purity of samples ranges from 60-70 % as estimated by immunostaining.

During isolation of CTCs, integrity of tumor cell membrane can be compromised. This technical limitation should be less problematic in evaluating the nuclear localized/DNA bound ER, PR, AR, and HSF1 proteins. We propose to evaluate these proteins in CTCs at baseline, Cycle 2 Day 8, Cycle 2 Day 9, and at time of progression. Exploratory comparisons of Arm A and Arm B will allow for assessment of the effect of fulvestrant plus Hsp90 inhibition versus fulvestrant alone. Amplification of EGFR and HER2 has been postulated as potential resistance mechanisms to endocrine therapy. EGFR and HER2 will also be assessed at the timepoints listed above to explore potential correlates to upfront and acquired resistance.

9. STUDY CALENDAR

All subjects must sign an informed consent document prior to initiation of any study related procedures. The informed consent document must be signed within 30 days before randomization. Baseline laboratory evaluations must be completed within 14 days before randomization. Baseline measurements including scans must be completed within 28 days before randomization. QTc must be documented by 12-lead ECG within 28 days before randomization. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

All assessments must be performed prior to administration of any study medication. Ganetespib should be administered within ± 3 days of the protocol-specified date, unless otherwise noted. All study assessments should be performed within +/- 3 days of the protocol-specified date; however, for study assessments minor deviations in scheduling are allowed to account for holidays, vacations, etc. and will not be considered protocol violations. For patients initially assigned to Arm A, after cross-over to Arm C (fulvestrant + ganetespib), fulvestrant dosing may occur up to +/-7 days in order to allow "synchronization" of the fulvestrant and ganetespib "Day 1".

Each study cycle should last ~28 days. Doses of ganetespib that are missed for any reason should not be considered "held," but should be considered "skipped". For example, if a patient is unable to receive Cycle 3 Day 8 (+/- 3 days) of treatment for any reason, the dose should be skipped and the patient should proceed on the appropriate calendar date to Cycle 3 Day 15 treatment. The purpose of this rule is to hold the cycle length reasonably constant in order to allow "synchronization" of the fulvestrant and ganetespib cycles (since it is unlikely that fulvestrant will be held for any reason). For the purpose of case report forms (CRF) and protocol charts, for patients receiving both fulvestrant and ganetespib, the cycle number should be assigned according to the ganetespib dosing (i.e., day 1 of the ganetespib cycle is considered day 1 of the cycle to be reported in the CRF). For patients receiving fulvestrant only, day 1 of fulvestrant dosing should be considered day 1 of the cycle.

Four study calendars are provided below:

A) Arm A—fulvestrant alone

B) Arm B—fulvestrant + ganetespib

C) Arm C— Patients initially assigned to Arm A, after crossover to fulvestrant + Ganetespib

D) Calendar of Events for Patients who are Taken Off Assigned Protocol Therapy for Reason Other than Progression or Death.

9.1 STUDY CALENDAR A

ARM A: Fulvestrant Alone—Initial Therapy

Note: The study calendar below describes initial study procedures for Arm A.

- Patients who progress on fulvestrant alone may cross over to Arm C, fulvestrant + ganetespib and should be followed at that time according to Study Calendar C, or they may elect to discontinue study treatment altogether.
- *Patients who discontinue fulvestrant for a reason other than progression should be followed at that time according to Study Calendar D.*
- **Patients who are taken off Arm A for reason other than progression and who are not followed on study calendar D for any reason, should have "off-treatment" evaluation and tests as described in the "At time of progression or off-treatment" column below. Patients are then followed for survival status as described in the "follow-up" column below.*

	Pre-Study	**C1 D1**	**C1 D15**	**C2 D1**	**C2 D8**	**C2 D9**	**C3 D1**	**Day 1 of each cycle**	**Every 2 cycles**	**At time of progression or off-treatment***	**Follow-Up**
Informed consent	X										
Confirm eligibility	X										
Demographics	X										
Medical History	X										
Concurrent medications	X	X	X	X			X	X		X	
Physical examination		X	X	X			X	X		X	
Vital signs[a]		X	X	X			X	X		X	
Height		X									
Weight		X								X	
ECOG performance status	X	X	X	X			X	X		X	
CBC w/diff, plts	X	X		X			X	X		X	
Serum chemistry[b]	X	X		X			X	X		X	
PT,PTT[c]											
B-HCG[d]	X										
CEA, CA27-29[e]		X					X[e]		X[e]	X	
12-lead EKG	X									X[f]	
Adverse event evaluation[g]	X	X	X	X			X	X		X	
Tumor measurements[h]	X								X[h]	X	
Bone scan[i]	X								X[i]	X	
Fulvestrant		X	X	X			X	X			
Research specimen collection (see section 9.5)	X	X			X	X				X	
Survival Status (see Section 5.1.16)											X q3months

a. Vital signs: heart rate, blood pressure, respiratory rate
b. Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin, calcium, magnesium, phosphorus
c. PT, PTT if clinically indicated prior to biopsy (-ies) per standard institutional guidelines.
d. In women of childbearing potential only (including premenopausal women with intact uterus and ovaries on GnRH agonist).
e. All patients should have baseline tumor marker measurement and tumor marker measurement at time of cross-over. Tumor markers are required at other timepoints only when elevated at baseline.
f. EKG required at time of progression only for patients intending to cross over to Arm C.
g. Baseline symptoms should be documented using the CTCAE v 4.0 grading scale. Adverse events should be documented on day 1 of each cycle, at each clinic visit, and at time of progression, using the CTCAE v 4.0 grading scale.
h. Radiologic imaging studies to evaluate tumor status should be done every 2 cycles +/- 7 days. However, patients who have been on-study for >1 year are permitted to have tumor imaging performed every 2-4 cycles. Contrast computed tomography (CT) (or without contrast if contraindicated) of chest and abdomen is preferred. Magnetic resonance imaging (MRI) of chest and abdomen is an acceptable alternative. Note: PET-CT scans are not allowed per protocol for primary assessment of response. PET-CT scans may be done according to clinical judgment to supplement CT or MRI scans in cases where results of the PET-CT may clarify or resolve a clinical question. If palpable/visible lesions (e.g. chest wall nodule, etc) are being used as target lesions, measurements and photographs should be taken as well.
i. A bone scan should be done at baseline and at time of cross-over in all patients. Patients with evaluable but non-measurable disease and with bone metastases should also undergo bone scan once every 2 cycles, unless all of the bony disease is encompassed within the area visualized by the restaging CT and/or MRI. Patients with bone only disease will be evaluated for progression but not for objective response. Patients with measurable disease by RECIST 1.1 need not undergo routine bone scans(with the exception of the baseline and time of cross-over scan) unless there is a clinical indication (example: new bone pain). Bone progression is defined as a bone event requiring intervention (e.g. surgery/radiation), the occurrence of a pathologic fracture or the appearance of new lytic lesions or other new bone destruction thought to be related to cancer by X-ray, MRI, or CT scan. Changes in bone scan should not be used to define progression. Disease “hot spots” detected on bone scan should be evaluated radiographically by X-ray, MRI, or CT scan to ascertain the presence of bone destruction versus a healing reaction. The appearance of new lesions on bone scan may constitute progressive disease if associated with clinical symptoms suggestive of disease progression. Patients with bone metastases will also be considered as having progressive disease if there are new and/or progressive lesions in non-bone sites.

9.2 STUDY CALENDAR B

Arm B: Fulvestrant + Ganetespib

Note: The study calendar below describes study procedures for Arm B.

- *Patients who discontinue ganetespib and/or fulvestrant for a reason other than progression should be followed at that time according to Study Calendar D.*

- **Patients who are taken off Arm B for reason other than progression and who are not followed on study calendar D for any reason, should have "off-treatment" evaluation and tests as described in the "At time of progression or off-treatment" column below. Patients are then followed for survival status as described in the "follow-up" column below.*

	Pre-Study	**C1 D1**	**C1 D8**	**C1 D15**	**C2 D1**	**C2 D8**	**C2 D9**	**C2 D15**	**C3 D1**	**Day 1 of each cycle**	**D8 and D15 of each cycle (cycle 3 forward)**	**Every 2 cycles**	**At time of progression or off-treatment***	**Follow-Up**
Informed consent	X													
Confirm eligibility	X													
Demographics	X													
Medical History	X													
Concurrent medications	X	X	X	X	X				X	X			X	
Physical exam		X	X	X	X				X	X			X	
Vital signs[a]		X	X	X	X	X		X	X	X	X		X	
Height		X												
Weight		X	X	X	X	X		X	X	X	X		X	
ECOG perf. status	X	X	X	X	X				X	X			X	
CBC w/diff, plts	X	X	X	X	X	X		X	X	X	X		X	
Serum chemistry[b]	X	X	X	X	X	X		X	X	X	X		X	
PT, PTT[c]														
B-HCG[d]	X													
CEA, CA27-29[e]		X							X[e]			X[e]	X	
12-lead ECG	X	X[f]			X[f]					X[f]				
Adverse event evaluation[g]	X	X	X	X	X				X	X			X	
Tumor measurements[h]	X											X[h]	X	
Bone scan[i]	X											X		
Fulvestrant		X		X	X				X	X				
Ganetespib		X	X	X	X	X		X	X	X	X			
Research Specimen Collection (see section 9.5)	X	X				X	X						X	
Survival Status (see Section 5.1.16)														X q3 months

a. Vital signs: heart rate, blood pressure, respiratory rate
b. Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin, calcium, magnesium, phosphorus
c. PT, PTT if clinically indicated prior to biopsy (-ies) per standard institutional guidelines
d. In women of childbearing potential only (including premenopausal women with intact uterus and ovaries on GnRH agonist).
e. All patients should have baseline tumor marker measurement. Tumor markers are required at other time points only when elevated at baseline.
f. For Arms B and C only: For patients at low risk for torsades de pointes an EKG must be performed Cycle 1 Day 2 (approximately 24 hours post Cycle 1 Day 1 ganetespib dose) and pre-dose (ganetespib) Day 1 of all subsequent cycles. If a Day 1 pre-dose QTc > 470ms then a repeat EKG is required approximately 24 hours post-ganetespib dose. Additional EKGs are required for patients at moderate to high risk for torsades de pointes. See section 5.6 for details.
g. Baseline symptoms should be documented using the CTCAE v 4.0 grading scale. Adverse events should be documented on day 1 of each cycle, at each clinic visit, and at time of progression.
h. Radiologic imaging studies to evaluate tumor status should be done every 2 cycles +/- 7 days. However, patients who have been on-study for >1 year are permitted to have tumor imaging performed every 2-4 cycles. Contrast computed tomography (CT) (or without contrast if contraindicated) of chest and abdomen is preferred. Magnetic resonance imaging (MRI) of chest and abdomen is an acceptable alternative. Note: PET-CT scans are not allowed per protocol for primary assessment of response. PET-CT scans may be done according to clinical judgment to supplement CT or MRI scans in cases where results of the PET-CT may clarify or resolve a clinical question. If palpable/visible lesions (e.g. chest wall nodule, etc) are being used as target lesions, measurements and photographs should be taken as well.
i. A bone scan should be done at baseline and at time of cross-over in all patients. Patients with evaluable but non-measurable disease and with bone metastases should also undergo bone scan once every 2 cycles, unless all of the bony disease is encompassed within the area visualized by the restaging CT and/or MRI. Patients with bone only disease will be evaluated for progression but not for objective response. Patients with measurable disease by RECIST 1.1 need not undergo routine bone scans(with the exception of the baseline and time of cross-over scan) unless there is a clinical indication (example: new bone pain). Bone progression is defined as a bone event requiring intervention (e.g. surgery/radiation), the occurrence of a pathologic fracture or the appearance of new lytic lesions or other new bone destruction thought to be related to cancer by X-ray, MRI, or CT scan. Changes in bone scan should not be used to define progression. Disease “hot spots” detected on bone scan should be evaluated radiographically by X-ray, MRI, or CT scan to ascertain the presence of bone destruction versus a healing reaction. The appearance of new lesions on bone scan may constitute progressive disease if associated with clinical symptoms suggestive of disease progression. Patients with bone metastases will also be considered as having progressive disease if there are new and/or progressive lesions in non-bone sites.

9.3 STUDY CALENDAR C

ARM C: Patients Assigned Arm A, After Cross-Over to Fulvestrant + Ganetespib

Note: Patients who progress on fulvestrant alone may cross over to fulvestrant + ganetespib. ***The first dose of ganetespib should be given only AFTER central confirmation of progression but within 2 weeks of documented progression on fulvestrant alone.*** Cycle number will re-set to Cycle 1 and be captured in case report forms as post-crossover treatment. Fulvestrant may be given +/- 7 days of schedule in order to allow "synchronization" of fulvestrant and ganetespib cycles.

- **Patients who are taken off Arm C for reason other than progression and who are not followed on study calendar D for any reason, should have "off-treatment" evaluation and tests as described in the "At time of progression or off-treatment" column below. Patients are then followed for survival status as described in the "follow-up" column below.*

	C1 D1	C1 D8	C1 D15	C2 D1	C2 D8 and C2D15	C2 D8 and C2 D9	C3D1	Day 1 of each cycle	D8 and D15 of each cycle	Every 2 cycles	At time of progression or off-treatment*	Follow-Up
Concurrent medications	X	X	X	X			X	X			X	
Physical examination	X	X	X	X			X	X			X	
Vital signs[a]	X	X	X	X	X		X	X	X		X	
Weight	X	X	X	X	X		X	X	X			
ECOG performance status	X			X			X	X			X	
CBC w/diff, plts	X	X	X	X	X		X	X	X		X	
Serum chemistry[b]	X	X	X	X	X		X	X	X		X	
PT, PTT[c]												
CEA, CA27-29							X[d]			X[d]	X	
12-lead EKG	X[h]			X[h]				X[h]				
Adverse event evaluation[e]	X	X	X	X			X	X			X	
Tumor measurements[f]										X[f]	X	
Bone scan[g]										X[g]	X[g]	
Fulvestrant	X			X			X	X				
Ganetespib	X	X	X	X	X		X	X	X			
Research Specimen Collection (see section 9.5)											X	
Survival Status (see Section 5.1.16)												X q3 months

a. Vital signs: heart rate, blood pressure, respiratory rate
b. Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin, calcium, magnesium, phosphorus

c. Patients receiving ganetespib and who are on warfarin should have PT-INR checked at baseline, 1 week, 2 weeks, and 4 weeks after start of ganetespib and as clinically indicated thereafter. Patients not on warfarin are not required to have PT, PTT checked at any time, except if clinically indicated prior to a biopsy.
d. Tumor markers should be drawn at off-study. Other timepoints are required only when elevated at time of cross-over.
e. Adverse events should be documented on day 1 of each cycle, at each clinic visit, and at time of cross-over, using the CTCAE v 4.0 grading
f. Radiologic imaging studies to evaluate tumor status should be done every 2 cycles +/- 7 days. However, patients who have been on-study for >1 year are permitted to have tumor imaging performed every 2-4 cycles. Contrast computed tomography (CT) (or without contrast if contraindicated) of chest and abdomen is preferred. Magnetic resonance imaging (MRI) of chest and abdomen is an acceptable alternative. Note: PET-CT scans are not allowed per protocol for primary assessment of response. PET-CT scans may be done according to clinical judgment to supplement CT or MRI scans in cases where results of the PET-CT may clarify or resolve a clinical question. If palpable/visible lesions (e.g. chest wall nodule, etc) are being used as target lesions, measurements and photographs should be taken as well.
g. A bone scan should be done at baseline and at time of cross-over in all patients. Patients with evaluable but non-measurable disease and with bone metastases should also undergo bone scan once every 2 cycles, unless all of the bony disease is encompassed within the area visualized by the restaging CT and/or MRI. Patients with bone only disease will be evaluated for progression but not for objective response. Patients with measurable disease by RECIST 1.1 need not undergo routine bone scans(with the exception of the baseline and time of cross-over scan) unless there is a clinical indication (example: new bone pain). Bone progression is defined as a bone event requiring intervention (e.g. surgery/radiation), the occurrence of a pathologic fracture or the appearance of new lytic lesions or other new bone destruction thought to be related to cancer by X-ray, MRI, or CT scan. Changes in bone scan should not be used to define progression. Disease “hot spots” detected on bone scan should be evaluated radiographically by X-ray, MRI, or CT scan to ascertain the presence of bone destruction versus a healing reaction. The appearance of new lesions on bone scan may constitute progressive disease if associated with clinical symptoms suggestive of disease progression. Patients with bone metastases will also be considered as having progressive disease if there are new and/or progressive lesions in non-bone sites.
h. For Arms B and C only: For patients at low risk for torsades de pointes an EKG must be performed Cycle 1 Day 2 (approximately 24 hours post Cycle 1 Day 1 ganetespib dose) and pre-dose (ganetespib) Day 1 of all subsequent cycles. If a Day 1 pre-dose QTc > 470ms then a repeat EKG is required approximately 24 hours post-ganetespib dose. Additional EKGs are required for patients at moderate to high risk for torsades de pointes. See section 5.6 for details.

9.4 STUDY CALENDAR D

Calendar of Events for Patients who Discontinue Study Treatment for Reasons Other than Progression or Death.

As detailed in Section 5.6 and Section 5.8, participants will be removed from study when any of the following occur

- Disease progression,
- Start of new anti-cancer therapy, or
- Participant decides to withdraw from the study follow up procedures
- Subject death

Prior to removal from study, patients in Arm A who progress on fulvestrant alone may first cross over to the combination arm. Patients in Arm A who discontinue fulvestrant for any reason other than centrally confirmed progression or death, and who do not cross over should continue to be followed according to the study calendar below, **until evidence of disease progression, start of new anti-cancer therapy, withdrawal of consent to follow-up, or death, whichever occurs first.**

For patients in Arm B or Arm C, once ganetespib is discontinued for any reason, patients ***should not be removed from study*** **but instead should remain on fulvestrant and continue to be followed per protocol according the study calendar below, until evidence of disease progression, start of new anti-cancer therapy, withdrawal of consent to follow-up, or death, whichever occurs first . Patients who choose to discontinue fulvestrant without evidence of disease progression should also be followed per protocol, as specified below, until evidence of disease progression, start of new anti-cancer therapy, withdrawal of consent to follow-up, or death, whichever occurs first.**

	Every 4 weeks	Every 8 weeks	Off-Study	Follow-Up
Concurrent medications	X		X	
Physical examination	X		X	
Vital signs[a]	X		X	
ECOG performance status	X		X	
CBC w/diff, plts		X	X	
Serum chemistry[b]		X	X	
CEA, CA27-29			X[e]	
Adverse event evaluation[g]	X		X	
Tumor measurements[i]		X[i]	X	
Bone scan[j]		X	X	
Fulvestrant, if applicable (see above)	X			
Research Specimen Collection (see section 9.5)			X	

Survival Status (see section 5.1.16)				X q3 months

a. Vital signs: heart rate, blood pressure, respiratory rate
b. Serum chemistry: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose (random), total bilirubin, alkaline phosphatase, ALT, AST, albumin, calcium, magnesium, phosphorus
c. Patients receiving ganetespib and who are on warfarin should have PT-INR checked at baseline, 1 week, 2 weeks, and 4 weeks after start of ganetespib and as clinically indicated thereafter. Patients not on warfarin are not required to have PT, PTT checked at any time, except if clinically indicated prior to a biopsy.
d. In women of childbearing potential only.
e. All patients should have baseline tumor marker measurement. Tumor markers are required at other timepoints only when elevated at baseline.
g. Baseline symptoms should be documented using the CTCAE v 4.0 grading scale. Adverse events should be documented on day 1 of each cycle, at each clinic visit, and at time of cross-over, using the CTCAE v 4.0 grading scale.
i. Radiologic imaging studies to evaluate tumor status should be done every 2 cycles +/- 7 days. However, patients who have been on-study for >1 year are permitted to have tumor imaging performed every 2-4 cycles. Contrast computed tomography (CT) (or without contrast if contraindicated) of chest and abdomen is preferred. Magnetic resonance imaging (MRI) of chest and abdomen is an acceptable alternative. Note: PET-CT scans are not allowed per protocol for primary assessment of response. PET-CT scans may be done according to clinical judgment to supplement CT or MRI scans in cases where results of the PET-CT may clarify or resolve a clinical question. If palpable/visible lesions (e.g. chest wall nodule, etc) are being used as target lesions, measurements and photographs should be taken as well.
j. A bone scan should be done at baseline in all patients. Patients without bone metastases at baseline do not require additional bone scans as part of this study, unless clinically indicated on the basis of symptoms, laboratory evaluation, etc. Patients with evaluable but non-measurable disease and with bone metastases should also undergo bone scan once every 2 cycles, unless all of the bony disease is encompassed within the area visualized by the restaging CT and/or MRI. Patients with bone only disease will be evaluated for progression but not for objective response. Patients with measurable disease by RECIST 1.1 need not undergo routine bone scans(with the exception of the baseline and time of cross-over scan) unless there is a clinical indication (example: new bone pain). Bone progression is defined as a bone event requiring intervention (e.g. surgery/radiation), the occurrence of a pathologic fracture or the appearance of new lytic lesions or other new bone destruction thought to be related to cancer by X-ray, MRI, or CT scan. Changes in bone scan should not be used to define progression. Disease “hot spots” detected on bone scan should be evaluated radiographically by X-ray, MRI, or CT scan to ascertain the presence of bone destruction versus a healing reaction. The appearance of new lesions on bone scan may constitute progressive disease if associated with clinical symptoms suggestive of disease progression. Patients with bone metastases will also be considered as having progressive disease if there are new and/or progressive lesions in non-bone sites.

9.5 SPECIMEN SUBMISSION REQUIREMENTS

The following specimens are to be submitted to the indicated lab or location. Refer to the information in sections 9.5.1-9.5.3 for specimen collection scheduling, billing, processing, and shipping instructions.

Specimen Type	Time Point							Shipping Condition	Ship to[g]
	Pre-Study	C1 D1[a]	C2 D8	C2 D9	At time of progression[c]	Off-treatment without progression[c]	Off Study[c]		
Archival blocks (Section 9.5.1)	X							Ambient temperature	Danielle Gore
Optional biopsy (Section 9.5.2)	X				X (Arms A & B only)		X	OCT: Frozen (dry ice) Trizol (FNA): Ambient RPMI (FNA): ice/ice pack	Danielle Gore
Required biopsy (Section 9.5.2)				X (Arms A&B only)					
10 mL Streck tube (Section 9.5.3)		X[b] (Arms A & B only)			X[f]	X	X	Ambient temperature	Danielle Gore
10 mL Lavender top tube (Section 9.5.3)		X[b] (Arms A & B only)						Ambient temperature	Danielle Gore
PBMC collection (2 PAXgene tubes) (Section 9.5.3)		X (Arms A & B only)	X (Arms A & B only)	X (Arms A & B only)	X	X	X	Frozen (dry ice)	Luke Whitesell/ Whitehead Institute
Circulating tumor cells (2- 7.5 mL CellSave tubes) (Section 9.5.3)		X (Arms A & B only)	X (Arms A & B only)	X (Arms A&B only)	X	X	X	Ambient temperature	Danielle Gore

a. Samples may be collected at any time after documentation of written consent and confirmation of protocol eligibility, but BEFORE the first dose of protocol treatment. The best time to obtain this is on Cycle 1 Day 1.
b. If baseline sample collection is missed for any reason, the sample should be drawn at a future appointment.
c. Off-study blood samples should be drawn within 30 days of off-study. Time of progression blood samples may be counted as the "off study" blood sample if the patient is coming permanently off-study. Time of progression blood samples are drawn if patient progresses on Arms A or B (1st Regimen) or Arm C (2nd Regimen).

Coded laboratory specimens will be stored in the Tumor Bank of the DFCI. These specimens will become the property of DFCI. Patients will be informed that their specimens may be used for research by investigators at DF/HCC and Whitehead Institute. Specimens will be identified with a sample ID number; all patient identifying material will be removed.

9.5.1 Archival tissue

Confirmation of availability of archival blocks (or unstained slides) of primary and/or metastatic tissue is required prior to eligibility. Tissue blocks should be sent to Dana-Farber Cancer Institute within in 2 weeks of the start of protocol therapy. If blocks cannot be submitted, 10-20 unstained slides are acceptable. If unstained slides will be sent as an alternative, those slides should not be sent until specifically requested by the DFCI study coordinator.

Complete a *DFCI 11-477 Specimen Requisition (Archival Tissue Blocks/Slides and CTC Samples)* form (Appendix J) and ship archival tissue blocks/slides at ambient temperature to:

Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Email Danielle Gore with the sample information and tracking information the day before shipping specimens dgore@partners.org

If requested, blocks will be returned to sites after the completion of the specimen analysis.

9.5.2 Tissue Biopsies

Tissue specimens will be collected from tumor lesions using standard institutional procedures. See Appendix B for standard operating procedures of biopsies through the Brigham & Women's Hospital interventional radiology service. Treating investigators should use their best judgment in approaching appropriate patients for optional biopsies at the indicated time points.

Tumor tissue may be collected at the following time points on this study:

- Baseline (OPTIONAL)
- Cycle 2 Day 9 (REQUIRED for patients with biopsy-accessible disease)
- Time of cross-over (Arm A only)—(OPTIONAL)
- Off-study/time of progression (OPTIONAL)

Tissue Biopsy Guidelines

- **The cost of the on-treatment (Cycle 2) research biopsy will be covered by the study.** For the baseline, time of cross-over, and off-study biopsies, the research biopsy will be covered by the study if it is being done for research only. However, at these time points, it is recognized that biopsies might be considered to aid in clinical management (for example, to re-assess ER, PR, and HER2 status). In the case of a clinically indicated biopsy (with an additional "research pass"), the cost of the biopsy will be charged to the patient and/or her insurance company.
- **For Arm A patients**: The Cycle 2 Day 9 biopsy may occur +/- 3 days, and on the same day as the Cycle 2 Day 9 research blood draw. If the Cycle 2 Day 9 biopsy is missed (i.e. due to scheduling problems, procedure cancellation, etc), then it should be performed instead on Cycle 2 Day 16 +/- 3 days, and the Cycle 2 research blood tests should be rescheduled to occur around the delayed biopsy (i.e. Cycle 2 Day 15 and Cycle 2 Day 16).
- **For Arm B patients:** Obtain the Cycle 2 Day 9 biopsy 18-48 hours after the Cycle 2 Day 8 dose of ganetespib. If the Cycle 2 Day 8 dose is held for any reason (e.g. for toxicity), the Cycle 2 biopsy should be performed between 18-48 hours after the Cycle 2 Day 15 dose of ganetespib.
- Patients who undergo an attempted Cycle 2 research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are not required to undergo a repeat biopsy in order to continue on protocol.

The amount of tissue collected will follow the guidelines listed below. If a patient has more than one site of disease, only one site needs to be biopsied, and the site is left to the discretion of the patient and her treating physician. Core biopsies are preferred over fine needle aspirates when both are technically feasible. However, fine needle aspirates are acceptable and may be used.

Breast:

- A goal of 3-6 core biopsy (or FNA specimens) will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass

Skin/Chest wall:

- A goal of two 5-mm punch biopsies

Lymph node/Soft tissue:

- A goal of 3-6 core biopsy (or FNA) specimens will be obtained using an 18-gauge needle (or fine needle).

Liver:

- A goal of 3-6 core biopsy (or FNA) specimens will be obtained using an 18-gauge needle (or fine needle)

Lung:

- Because of the risk of pneumothorax associated with lung biopsies, additional care should be taken in discussing the case with radiology and/or thoracic surgery prior to proceeding with a biopsy and the number of research samples attempted adjusted per clinical judgement. If other more easily accessible sites are available, they should be prioritized for biopsy. Either core biopsy or fine needle aspirations acceptable/allowable
- A goal of 3 core biopsy specimens will be obtained using an 18-gauge needle (or fine needle)
- A goal of 3-6 FNA passes will be obtained using standard institutional guidelines for needle gauge. As with all other biopsy sites, less than the goal amount is allowed and subject to the clinical judgment of the physician performing the procedure.

Bone:

- Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a patient has another accessible site of disease (e.g., skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 1-3 core biopsy specimens will be obtained using an 11 to 13-gauge needle.

Pleural Fluid:

- A goal of 500 cc of pleural fluid will be obtained with a standard thoracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

Ascites fluid:

- A goal of 500 cc of ascites fluid will be obtained with a standard paracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

Please note that the above are guidelines for the amount of tissue to be obtained at the baseline biopsy, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure. If ascites or pleural fluid is to be used as the investigational biopsy specimen, consideration should be given to confirming the malignant nature of the ascites or pleural fluid prior to study entry.

Risks of Research Biopsies and Procedures for Minimizing Risk

Potential risks according to site are:

Breast (core biopsy/FNA):
- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.

Skin/chest wall (punch biopsy):
- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, or infection

Lymph node or soft tissue (core needle biopsy/FNA):
- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.
- Additional risks may be present if i.v. conscious sedation is required.

Liver (core needle biopsy/FNA):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs
- Additional risks may be present if i.v. conscious sedation is required.

Lung (FNA):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs, lung collapse
- Additional risks may be present if i.v. conscious sedation is required.

Bone (core needle biopsy/FNA):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs
- Additional risks may be present if i.v. conscious sedation is required.

Pleural fluid (thoracentesis):
- Likely: local discomfort and minor bleeding

- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs

Ascites fluid (paracentesis):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs

In order to minimize the risk of a biopsy, only qualified personnel will perform these procedures. Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain a separate procedure consent. Patients will be evaluated for co-morbidities or concomitant medications that may increase the risk of potential complications. For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT or ultrasound will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. After lymph node biopsies, patients will be observed a minimum of 2 hours (range 2-4 hours) after the procedure, or according to standard institutional guidelines. After liver biopsies, patients will be observed a minimum of 4 hours (range 4-6 hours) after the procedure, or according to standard institutional guidelines. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

Risks of Anesthesia

Local Anesthesia
All biopsy procedures require local anesthesia using xylocaine (Lidocaine®) or related compounds. There is a small risk of an allergic reaction associated with these drugs.

In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation
Certain biopsy procedures, such as lymph node, liver, or bone biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and to respond appropriately to physical stimulation and verbal commands.

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 patients undergoing IVCS during upper gastrointestinal endoscopies, the rate of

immediate cardiopulmonary events was 2 in 1000. The 30-day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia.

In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (M.D. or R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Patients with active cardiac disease are excluded from this study. No local anesthetic spray to the oropharynx will be necessary, given that endoscopy is not a planned procedure. Following the procedure, patients will be observed closely in the recovery room for a minimum of 2 hours.

General Anesthesia

Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed *are not permitted* on this protocol, unless they are being done for clinical reasons, and excess tissue that otherwise would have been discarded is then banked for the purpose of this protocol.

Tissue Biopsy Processing

Following the biopsy of a specimen, all tissue cores will be snap-frozen in OCT compound intra-operatively. This sample handling is optimal for obtaining high quality total RNA from human tumor tissue for microarray studies. After the biopsy is performed, the tissue mass is placed on a dry sterile gauze and the tumor tissue is separated using forceps. Two pieces (cores) of tumor tissue are placed on a pre-frozen bed of OCT in each cassette, typically ending up with 3 cassettes per biopsy (the last cassette will contain many small pieces of tumor tissue). Cassettes are then filled with OCT, completely covering the tissue and limiting the amount of bubbles (forceps can be used to pop any bubbles). Tissue must be frozen immediately by placing cassettes on dry ice in a biohazard cooler; freezing of samples in OCT with dry ice takes about 10 minutes freezing time. Once samples are frozen, place in plastic bag; label bag with date, protocol number, patient number, patient initials, and number of biopsy samples included. Store in -80^{o}C freezer until the time of shipment.

For patients who undergo fine needle aspiration (FNA) rather than core biopsy or punch biopsy 3 passes should be collected. The first pass should be evacuated and rinsed directly into 2mL of room temperature Trizol to collect the RNA; a second pass should be evacuated and rinsed directly into another 2mL of room temperature Trizol to collect the DNA; the last pass should be evacuated and rinsed directly into 10-20mL of RPMI and prepared as a cell block.

For patients who have pleural fluid or ascites collected, samples should be collected in a sterile vacutainer. The fluid sample should be split into three equal aliquots. One aliquot should be spun down into a pellet and snap frozen in an ETOH/dry ice bath or in liquid N2. One aliquot should be fixed and processed as a standard cell block. One aliquot should be spun down and transferred into Cell Save preservative tubes (7.5 mL) in the research lab. Fluid samples can be transported and stored at room temperature up to 72 hours before processing. Do not refrigerate or freeze the sample. Samples must be processed within 72 hours of collection, but best results are obtained if the sample is processed as soon as possible.

All samples will be anonymized by assigning a unique sample ID number prior to use. Histopathologic review will be performed at DFCI/BWH on hematoxylin and eosin-stained sections taken from two tissue planes and study samples will be obtained from the tissue sandwiched in between these two sections. This will assure that the histopathologic review is representative of the specimens that we actually process. If a sample is sufficiently large, multiple aliquots of at least 250 mg/piece will be frozen in separate tubes. These will be labeled to identify that the aliquots came from the same original specimen.

Tissue biopsy collection and processing materials are not provided by the study. Coded laboratory specimens will be stored in the Tumor Bank of the DF/HCC. These specimens will become the property of DF/HCC. In cases where the research sample is forfeited for clinical purposes, this sample will be flagged in spreadsheet to indicate it can be used for research purposes if there is any left over after clinical use.

Please see Appendix C for additional processing details specific to the laboratories at Dana Farber Cancer Institute and Brigham and Women's Hospital.

Tissue Biopsy Shipping

Complete a *DFCI 11-477 Specimen Requisition (Biopsy/Research Blood)* form (Appendix K) and ship all biopsies samples to:

Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Email Danielle Gore with the sample information and tracking information the day before shipping specimens: dgore@partners.org

Shipping note: Frozen and ambient specimens obtained and shipped on the same day to Danielle Gore (e.g., Progression or Off Study Biopsy Specimens, Streck Tubes, and Circulating Tumor Cells) may be placed in a combination shipping box which contains separate compartments for frozen and ambient samples. If a combination shipping box is not available, two shipping boxes should be used. Frozen samples shipped to Luke Whitesell/Whitehead Institute obtained on the same day as these specimens must always be placed in a separate shipping box.

9.5.3 Blood Sample Collection

Blood will be collected at the indicated time points to be processed and banked in the DF/HCC Clinical Trials Core laboratory for future research purposes. Blood samples are collected four ways:

- Streck Tubes
- Lavender Top Tubes
- PBMC/PAXgene Tubes
- Circulating Tumor Cells/CellSave tubes

These samples will be used to look at DNA, RNA and protein in future studies. Refer to the details below for scheduling, collection, processing, and shipping details.

Streck Tubes

One 10 mL Streck tube will be collected and processed at baseline and time of progression (all study Arms) for evaluation of cell free circulating DNA (cfDNA). Tubes are provided by the Sponsor as needed.

- If the baseline sample is missed for any reason, it should be drawn at a future appointment.
- **For Arm A patients:** The Cycle 2 Day 8 sample may be drawn +/- 3 days. Draw the Cycle 2 Day 9 research blood 18-48 hours after the Cycle 2 Day 8 research blood draw and on the same day as the cycle 2 research biopsy. If the Cycle 2 Day 9 biopsy is missed (i.e. due to scheduling problems, procedure cancellation, etc), then it should be performed instead on Cycle 2 Day 16 +/- 3 days, and the Cycle 2 research blood tests should be rescheduled to occur around the delayed biopsy (i.e. Cycle 2 Day 15 and Cycle 2 Day 16). If a patient on Arm A does not undergo the Cycle 2 biopsy, then the Cycle 2 Day 9 blood draw is not required. However, the patient is still required to undergo the Cycle 2 Day 8 research blood draw.
- **For Arm B patients:** Draw Cycle 2 Day 8 research blood 2-4 hours after ganetespib administration. Draw Cycle 2 Day 9 research blood 18-48 hours after ganetespib administration and on the same day as the cycle 2 research biopsy. The cycle 2 Day 9 research blood draw is required for all Arm B patients, including patients who do not undergo the cycle 2 research biopsy. If the Cycle 2 Day 8 dose is held for any reason

(e.g. for toxicity), or if the Cycle 2 Day 8 biopsy is instead rescheduled to Cycle 2 Cay 16, then draw the Cycle 2 research blood 2-4 hours after the Cycle 2 Day 15 dose of ganetespib and again 18-48 hours after the Cycle 2 Day 15 dose of ganetespib (i.e. on the same day as the cycle 2 research biopsy).

Complete a *DFCI 11-477 Specimen Requisition (Biopsy/Research Blood)* form (Appendix K). Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate results. Ship within 24 hours of collection at ambient temperature overnight to:

Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Email Danielle Gore with the sample information and tracking information the day before shipping specimens: dgore@partners.org

Tube precautions:

- If samples cannot be shipped within 24 hours of collection, contact DFCI. DO NOT FREEZE OR REFIDGERATE TUBES as this could result in cfDNA breakage.
- Do not use tubes after expiration date.
- Fill the tube completely; overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytic results.

See Appendix F for additional processing details specific to the laboratories at Dana Farber Cancer Institute.

Shipping note: Streck tubes samples are sent Ambient. Frozen and ambient specimens obtained and shipped on the same day to Danielle Gore (e.g., Progression or Off Study Biopsy Specimens, Streck Tubes, and Circulating Tumor Cells) may be placed in a combination shipping box which contains separate compartments for frozen and ambient samples. If a combination shipping box is not available, two shipping boxes should be used. Frozen samples shipped to Luke Whitesell/Whitehead Institute obtained on the same day as these specimens must always be placed in a separate shipping box.

Lavender top tube

One 10 mL lavender top tube will be collected at baseline in order to extract germline DNA to be used as normal DNA reference for tumor tissue-based studies. If the sample is missed for any reason, it should be drawn at a future appointment. These collection tubes are provided by the Sponsor as needed.

Complete a *DFCI 11-477 Specimen Requisition (Biopsy/Research Blood)* form (Appendix K) and ship lavender top tubes at ambient temperature to:

Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Email Danielle Gore with the sample information and tracking information the day before shipping specimens: dgore@partners.org

PBMC analyses

For PBMC analyses, blood will be drawn directly into two PAXgene blood RNA tubes (ref #762165) from Qiagen/BD. PAXgene tubes will be collected at baseline (C1D1), C2 D8 andC2D9 (Arms A & B only), at progression/end of treatment and end of study. The Cycle 2 Day 9 sample should be collected as close as possible to 24 hours after the Cycle 2 Day 8 dose and not more than 48 hours after the C2D8 dose.

- **For Arm A patients:** The Cycle 2 Day 8 sample may be drawn +/- 3 days. Draw the Cycle 2 Day 9 research blood 18-48 hours after the Cycle 2 Day 8 research blood draw and on the same day as the cycle 2 research biopsy. If the Cycle 2 Day 9 biopsy is missed (i.e. due to scheduling problems, procedure cancellation, etc), then it should be performed instead on Cycle 2 Day 16 +/- 3 days, and the Cycle 2 research blood tests should be rescheduled to occur around the delayed biopsy (i.e. Cycle 2 Day 15 and Cycle 2 Day 16). If a patient on Arm A does not undergo the Cycle 2 biopsy, then the Cycle 2 Day 9 blood draw is not required. However, the patient is still required to undergo the Cycle 2 Day 8 research blood draw.
- **For Arm B patients:** Draw Cycle 2 Day 8 research blood 2-4 hours after ganetespib administration. Draw Cycle 2 Day 9 research blood 18-48 hours after ganetespib administration and on the same day as the cycle 2 research biopsy. The cycle 2 Day 9 research blood draw is required for all Arm B patients, including patients who do not undergo the cycle 2 research biopsy. If the Cycle 2 Day 8 dose is held for any reason (e.g. for toxicity), or if the Cycle 2 Day 8 biopsy is instead rescheduled to Cycle 2 Cay 16, then draw the Cycle 2 research blood 2-4 hours after the Cycle 2 Day 15 dose of ganetespib and again 18-48 hours after the Cycle 2 Day 15 dose of ganetespib (i.e. on the same day as the cycle 2 research biopsy).

After the blood draw samples will sit at room temperature for two hours and then sit for 24 hours in a -20º C freezer. Following this, samples will be stored at -80º C until several samples have accumulated at which point samples will be shipped to Dr. Luke Whitesell's laboratory at the Whitehead Institute for Biomedical Research (Address below). A supply of PAXgene tubes will be shipped to participating sites as necessary. After the PBMC research blood draw, samples will sit at room temperature for two hours and then sit for 24 hours in a -20º C freezer. Following this, samples will be stored at -80º C until several samples have accumulated as tubes may be batched for shipping.

Complete a *DFCI 11-477 Specimen Requisition (PBMC Tubes)* form (Appendix K) and

ship PBMC tubes on dry ice to:

Luke Whitesell
Whitehead Institute
9 Cambridge Center
Cambridge, MA 02142

Circulating Tumor Cells

Blood samples for CTC analysis will be collected in two 7.5 mL (15mL total) CellSave tubes at baseline (C1D1), C2 D8 andC2D9 (Arms A & B only), at progression/end of treatment and end of study. The Cycle 2 Day 9 sample should be collected as close as possible to 24 hours after the Cycle 2 Day 8 dose and not more than 48 hours after the C2D8 dose.

- **For Arm A patients:** The Cycle 2 Day 8 sample may be drawn +/- 3 days. Draw the Cycle 2 Day 9 research blood 18-48 hours after the Cycle 2 Day 8 research blood draw and on the same day as the cycle 2 research biopsy. If the Cycle 2 Day 9 biopsy is missed (i.e. due to scheduling problems, procedure cancellation, etc), then it should be performed instead on Cycle 2 Day 16 +/- 3 days, and the Cycle 2 research blood tests should be rescheduled to occur around the delayed biopsy (i.e. Cycle 2 Day 15 and Cycle 2 Day 16). If a patient on Arm A does not undergo the Cycle 2 biopsy, then the Cycle 2 Day 9 blood draw is not required. However, the patient is still required to undergo the Cycle 2 Day 8 research blood draw.
- **For Arm B patients:** Draw Cycle 2 Day 8 research blood 2-4 hours after ganetespib administration. Draw Cycle 2 Day 9 research blood 18-48 hours after ganetespib administration and on the same day as the cycle 2 research biopsy. The cycle 2 Day 9 research blood draw is required for all Arm B patients, including patients who do not undergo the cycle 2 research biopsy. If the Cycle 2 Day 8 dose is held for any reason (e.g. for toxicity), or if the Cycle 2 Day 8 biopsy is instead rescheduled to Cycle 2 Cay 16, then draw the Cycle 2 research blood 2-4 hours after the Cycle 2 Day 15 dose of ganetespib and again 18-48 hours after the Cycle 2 Day 15 dose of ganetespib (i.e. on the same day as the cycle 2 research biopsy).

Samples should be shipped overnight to the can sit at room temperature for up to 72 hours but must be mixed immediately with fixative to help prevent clotting and transferred to SuperFrost Plus slide as cytospin preparation. Leftover slides will be banked at -20 degrees C for future processing. A supply of CellSave tubes will be shipped to participating sites as necessary.

See Appendix C for additional processing details specific to the laboratories at Dana Farber Cancer Institute and Brigham & Women's Hospital.

Complete a *DFCI 11-477 Specimen Requisition (Archival Tissue Blocks/Slides and CTC Samples)* form (Appendix J) and ship CTC tubes at ambient temperature to:

Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Email Danielle Gore with the sample information and tracking information the day before shipping specimens: dgore@partners.org

Shipping note: Frozen and ambient specimens obtained and shipped on the same day to Danielle Gore may be placed in a vertical combination shipping box (containing separate compartments for frozen and ambient samples). If a vertical combination shipping box is not available, two shipping boxes should be used. Frozen samples shipped to Luke Whitesell/Whitehead Institute obtained on the same day as these specimens must always be placed in a separate shipping box.

10. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable and/or non-measurable disease will be assessed by RECIST 1.1 criteria. For the purposes of this study, participants should be reevaluated every 8 weeks. In addition to a baseline scan, confirmatory scans and clinical measurements with calipers (if applicable) should also be obtained $\geq$ 4 weeks following initial documentation of an objective response.

10.1 Antitumor Effect– Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks. Confirmatory scans should be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) (Eisenhauer et al., 2009) guidelines. Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

10.1.1 Definitions

Evaluable for PFS. All participants randomized on this study are considered to be evaluable for PFS (whether or not they are eligible, have measurable disease, or receive protocol therapy).

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants who have measurable disease present at baseline and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

10.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

A lesion in a previously irradiated area is not eligible for measurable disease unless there is objective evidence of progression of the lesion prior to study enrollment. Lesions in previously irradiated areas must be clearly identified as such.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to < 15mm short axis, are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by *chest* x-ray. Nodes must have a short axis ≥ 15 mm. The short axis of target nodes should be included in the sum of the longest dimension of target lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. (If the patient has a sum of largest diameters of target lesions other than nodes that is zero, then the patient can be classified as having a CR if each target node has a short axis < 10 mm). Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the *soft tissue component* meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions. Lesions in previously irradiated areas or areas subject to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous

lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

FDG PET and PET/CT. The acquisition of FDG PET and FDG PET/CT scans should follow the NCI Guidelines for using FDG PET as an indicator of therapeutic response (L.K. Shankar, J.M. Hoffman, S. Bacharach, M.M. Graham, J. Karp, A.A. Lammertsma, S. Larson, D.A. Mankoff, B.A. Siegel, A. Van den Abbeele, J. Yap, D. Sullivan. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med, 47(6):901-903, 2006). Patients should avoid strenuous exercise and be on a low carbohydrate diet for 24 hours prior to the scan. Patients should fast for 4 hours or longer prior to the FDG injection and should have a serum glucose of less than 200 mg/dL at the time of FDG injection. A 10-20 mCi dose of FDG should be injected for typical adult patients. For longitudinal studies with multiple scans, particular attention should be paid to ensure consistent patient preparation and acquisition parameters between the follow-up scan and the baseline scan. When designing a study where PET scans are going to be utilized as one of the modalities to evaluate efficacy, it is important to consult with physicians in nuclear medicine in designing the appropriate criteria to be utilized.

Note: In this protocol, PET scans are not to be used for primary assessment of response. PET scans and/or PET-CT scans may be used to supplement CT or MRI scans when a clinical question as to response status still exists.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or

stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10.1.4 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph node must have reduction in short axis to < 10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study with at least a 5 mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease progression.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.5 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesions and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions* (new lesions must be > slice thickness) and/or unequivocal progression of existing non-target lesions. Overall level of substantial worsening that merits discontinuation of therapy. A useful test that can be applied when assessing non-targets for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.6 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response for when Confirmation is Required:
CR	CR	No	CR	≥4 wks confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/Not evaluated	No	PR	

SD	Non-CR/Non-PD/Not evaluated	No	SD	Documented at least once ≥4 wks from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as *"symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	NonCR/non-PD
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

10.1.7 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

10.1.8 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment until time of progression or death, whichever comes first. Patients who stop protocol-therapy (either ganetespib or fulvestrant or both) for a reason other than progression or death should be followed for progression, as detailed in Study Calendar D until the patient progresses, initiates alternative anti-cancer treatment, dies, or withdraws consent, whichever comes first. If the initiation of non-protocol anti-cancer treatment occurs first, then the patient's PFS will be censored at the time of last disease evaluation before start of the non-protocol anti-cancer treatment.

10.1.9 Overall Survival

Overall survival is defined as duration of time from start of treatment to death due to any cause, or censored on the date last known alive.

10.1.10 Response Review

The study will use the DF/HCC Tumor Imaging Metrics Core (TIMC) for central protocol measurements. For patients in Arm A, central confirmation of progression including DF/HCC TIMC review and confirmation by the overall study PI is required prior to crossover to Arm C and receipt of ganetespib.

10.2 Guidelines for Following Patients with Non-Measurable but Evaluable Disease

Patients with non-measurable but evaluable disease will be evaluated for progression but not for objective response. Bone progression is defined as a bone event requiring intervention (e.g., surgery/radiation), the occurrence of a pathologic fracture or the appearance of new lytic lesions or other new bone destruction thought to be related to cancer by X-ray, MRI, or CT scan. *Changes in bone scan alone should not be used to define progression.* Disease "hot spots" detected on bone scan should be evaluated radiographically by X-ray, MRI, or CT scan to ascertain the presence of bone destruction versus a healing reaction. The appearance of new lesions on bone scan may constitute progressive disease if associated with clinical symptoms suggestive of disease progression. In cases of uncertainty, FDG-PET may also be used to provide additional data; however, it should be noted that early "flare" responses may suggest response to therapy rather than progression (Ellis et al, J Am Med Assoc 2009). If a patient with bone-only disease progresses in another organ site per RECIST, that patient will also be counted as progressing.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

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.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6 for a listing of expected adverse events associated with the study agent(s).

Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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.

11.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Nancy Lin, MD
Tel: 617-632-2335
Fax: 617-632-1930
Pager: 617-632-3352, #42012
Email: nlin@partners.org

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

Nancy Lin, MD
Tel: 617-632-2335
Fax: 617-632-1930
Pager: #42012
Email: nlin@partners.org

The DF/HCC Principal Investigator will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

11.6 Reporting to the Food and Drug Administration (FDA)

The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.7 Reporting to Synta Pharmaceuticals, the Manufacturer

All adverse events meeting the regulatory (21CFR Subpart B §312.32 and Section 11.1.2 of this protocol) definition of serious must be reported to Synta Drug Safety.

Synta Drug Safety will provide SAE Report Forms and an SAE Completion Guideline for distribution by the Sponsor to each study site.

Serious adverse events should be reported to Synta within 24 hours of learning of the occurrence. Notification of SAEs may be made initially by **telephone at 1-866-255-0025**. All written reports and copies of source documents should be **fax**ed **to Synta Global Safety Surveillance at 1-888-975-2207**.

In addition copies of all FDA 3500A MedWatch forms submitted to FDA will be sent to Synta Global Safety Surveillance by the DF/HCC Overall Principal Investigator.

11.8 Reporting to Hospital Risk Management

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

11.9 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of scheduled response evaluation
Off Study Form	Within 14 days of being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

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.

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12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion. Please refer to Appendix G: Data Safety Monitoring Plan for additional details.

12.3.1 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix G.

The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
    - Title 21 Part 56 – Institutional Review Boards
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
    - Title 21 Part 312 – Investigational New Drug Application
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws
- DF/HCC research policies and procedures http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14. STATISTICAL CONSIDERATIONS

14.1 Background Data for Sample Size Justification

This study will enter patients who have either relapsed within 1 year of completing adjuvant endocrine therapy and/or who have progressed through at least one line of metastatic endocrine therapy. Patients who have previously received either fulvestrant or an HSP90 inhibitor are excluded. There is no limit on the number of prior lines of metastatic endocrine therapy. Up to one line of chemotherapy for metastatic disease is allowed.

The EFECT trial entered patients with ER-positive metastatic breast cancer who have progressed on a non-steroidal aromatase inhibitor.[4] Patients were randomized to receive fulvestrant versus exemestane. Approximately 60% of patients had received 2 or more prior lines of hormonal therapy (adjuvant + metastatic). Median time to progression was 3.7 months in both groups. Overall response rate was similar (7.4% v 6.7%; p=0.74). Clinical benefit rate (CR + PR + SD $\geq$ 6 months) was also similar (32.2% v 31.5%; p=0.85). The proportion of patients who came off study for a reason other than progression was not specifically reported in this study. However, at

the time of study reporting, 82% of the fulvestrant group and 87% of the exemestane group had experienced a defined progression event.

CALGB 40302 entered patients with ER-positive metastatic breast cancer who had previously been treated with an aromatase inhibitor, up to 2 lines of endocrine therapy for metastatic disease, and up to 1 prior line of metastatic chemotherapy.[5] Patients were randomized to fulvestrant with or without lapatinib. The study was terminated early for futility after the third planned interim analysis. Approximately half of patients had received prior tamoxifen; 28% of patients had bone-only disease. Median progression-free survival in the fulvestrant alone arm was 4.0 months.

14.2 Primary Clinical Objective and Sample Size Justification

Progression Free Survival (PFS): Progression-free survival is defined as time from randomization until time of progression or death, whichever comes first. Patients who stop protocol therapy (either fulvestrant or ganetespib or both) for a reason other than progression or death should continue to be followed for progression per Study Calendar D. For subjects who do not progress or die, PFS will be censored at the time of last tumor evaluation before initiation of alternative anti-cancer treatment.

Analysis Population: All randomized patients will be included in the analysis of PFS. The analysis will be conducted based on intention to treat (ITT). However, data imputation will not be used for PFS. So patients with no follow-up at all will be censored for PFS at the date of randomization.

If the interim safety analysis indicates that the starting dose of ganetespib should be decreased to 175 mg/m^2, these patients will be combined with the patients who received a starting dose of 200 mg/m^2 in the ITT analysis. That is, all randomized patients will be included in the ITT analysis, regardless of starting dose and regardless of whether or not treatment was stopped early for any reason.

Power Calculation

The primary objective of this randomized, phase II study is to investigate whether ganetespib, when given in combination with fulvestrant, improves progression-free survival (PFS) compared to fulvestrant alone, in patients with ER-positive, metastatic breast cancer. This study will have a 1:2 randomization ratio: 24 patients will be randomized to Arm A (fulvestrant alone) and 47 patients will be randomized to Arm B (fulvestrant + ganetespib), for a total of 71 randomized patients. The expected accrual rate is ~3.5 to 4.5 patients per month over a period of ~20 months.

As this is an exploratory phase II study, no interim efficacy analyses are planned and there are no early stopping rules for efficacy (or lack of efficacy) in this study.

The primary comparison will be a one-sided log rank test of whether PFS is longer in patients randomized to Arm B (fulvestrant + ganetespib) than those randomized to Arm A (fulvestrant).

A maximum of 20% of patients (n=14) on this trial may be entered on the strata for patients with non-measurable but evaluable disease (a category which includes but is not limited to patients with bone-only disease). The primary comparison will not be stratified by type of disease (measurable or non-measurable) because the stratification is being used as a mechanism to limit the number of patients with only non-measurable disease.

Based upon data from the EFECT trial, we further assume that up to 11 patients (15%) may be censored for PFS because of being lost to follow-up or receiving non-protocol anti-cancer therapy before they have progressed or died, and that this probability is not different in the two arms.[4] To be conservative, we assume that this censoring occurs very close to the time that the patient enters the study, so the effective total sample size will be 60, with 20 patients randomized to fulvestrant alone and 40 patients randomized to fulvestrant + ganetespib.

Assuming that PFS has an exponential distribution, and that the true median PFS on Arm A (fulvestrant alone) is 4.0 months, and that accrual takes 20 months (3.5 patients per month), there will be 80% power to detect a prolongation of true median PFS to 8.0 months in Arm B (fulvestrant + ganetespib), using a one-sided 0.05 log-rank test if the patients are followed for 20 months after accrual ends (so, 40 months after accrual starts). If accrual takes only 15 months (4.5 patients per month) and follow up extends to 22 months after accrual ends, there will also be 80% power. If the true median PFS on the single agent arm is longer than 4 months, the power calculations will still be correct as long as the ratio of medians remains the same (e.g. true medians of 5 and 10 months will have the same power as the true medians of 4 and 8 months, provided that the patients are followed long enough to have the same number of expected PFS events, which is 57).

14.3 Interim Safety Analysis

There are no phase I data with the combination of fulvestrant and ganetespib. Based upon the observed toxicities to date, mechanism of action, and drug metabolism, no additive or synergistic toxicities are anticipated. However, an interim safety analysis will be conducted among the first 10 patients randomized to Arm B (fulvestrant + ganetespib) and who receive at least one dose of ganetespib. No interim safety analysis will be conducted in patients randomized to Arm A, as fulvestrant is an approved agent in this setting with a very well-described toxicity profile. Accrual to the study will be placed on hold while the interim safety analysis is being conducted and results of the interim safety analysis will be submitted for review and approval by the DF/HCC IRB prior to re-opening the study for continued accrual.

DLT is based on the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE v4.0). DLT refers to toxicities experienced during the first cycle of treatment. A DLT will be defined as follows:

- Grade 3 or higher hematologic or non-hematologic toxicity thought likely or definitely related to protocol therapy will be counted as a DLT, *with the exception* of:

- Grade 3 diarrhea that responds to maximal supportive measures. If grade 3 diarrhea is persistent > 7 days despite maximal supportive measures, it will be counted as a DLT
- Grade 3 fatigue
- Grade 3 hot flashes
- Grade 3 electrolyte disturbance, unless persistent > 7 days despite maximal supportive measures.

All DLTs will be adjudicated by the study Principal Investigator and shared with the Synta medical monitor.

If more than 2 of the first 10 patients randomized to Arm B who receive some ganetespib have a DLT during the first cycle of therapy, study accrual will be temporarily suspended and the events will be adjudicated. Results will be shared with patients already enrolled on study, but they will be allowed to continue on therapy.

With this design, the probability of stopping accrual at or before 10 patients is 0.18 if the true DLT rate is 15%, 0.68 if the true DLT rate is 20%, and 0.90 if the true DLT rate is 45%.

If more than 2 patients of the first 10 have confirmed DLTs, the study will then re-open at Dose Level -1. If more than 2 of the first 10 patients randomized to arm B at Dose Level -1, study accrual will be temporarily suspended and the events will be adjudicated. If the events are confirmed by the Principal Investigator to be true DLTs, the study will close permanently to new accrual. Results will be shared with patients already enrolled on study, but they will be allowed to continue on therapy.

With this design, the probability of stopping accrual at or before 10 patients at Dose Level -1 is 0.18 if the true DLT rate is 15%, 0.68 if the true DLT rate is 20%, and 0.90 if the true DLT rate is 45%.

14.4 Analysis of Secondary Clinical Endpoints

Safety and toxicity: All patients who received at least one dose of protocol therapy (fulvestrant, ganetespib, or both) will be included in the safety/toxicity analysis. Toxicity will be graded according to NCI CTCAE, Version 4.0. The proportion of patients who experience each type and grade of toxicity will be presented in tabular form. Toxicity will be described separately in Arm A (pre-crossover), Arm C (post-crossover), and Arm B.

Objective Response Rate: All patients with measurable disease at baseline will be included in the analysis of response rate. Response will be assessed using RECIST 1.1, as described in Section 10. The response rate will be calculated separately in Arms A, B, and C. Response rates in Arm A and Arm B will be compared using a one-sided Fisher exact test. An intent to treat analysis will be used, so all patients randomized in the measurable disease stratum will be used in the

analysis; patients with no follow-up response evaluation will be assumed to be non-responders. Assuming that 57 patients are entered in the measurable disease stratum, there will be 81% power to decide that the ganetespib arm has a better response rate if the true response rates on the two arms are as different as 36% and 5%, or 44% and 10%.

Clinical benefit rate: Clinical benefit will be defined as CR + PR + SD>/= 6 months. All patients will be included in the analysis of clinical benefit. CBR will be calculated separately in Arms A and B, and compared using a one-sided Fisher exact test. All patients randomized on study will be used in this analysis, and patients with no follow-up response evaluation will be assumed not to have clinical benefit. With 71 patients randomized, there will be 80% power to decide that the ganetespib arm has a better clinical benefit rate if the true benefit rates on the two arms are as different as 63% and 30%, or 73% and 40%.

Overall survival: Overall survival is defined as duration of time from randomization to death due to any cause, or censored on the date last known alive. All randomized patients will be included in the analysis of OS. OS will be estimated separately in Arm A and Arm B. OS will be analyzed at the same time as the final analysis of PFS, although it is acknowledged that the percent of survival censoring at that time will be larger than the percent of PFS censoring, and this will limit the power to detect survival differences between the arms.

14.5 Analysis of Correlative Endpoints

The proposed studies of whether any markers are predictive or prognostic are exploratory and primarily hypothesis-generating in nature. For this reason, there will be no adjustment for multiple comparisons.

14.5.1 HSF1 and Outcome

The primary correlative objective is to test whether high nuclear expression of HSF1 nuclear localization (as assessed in archival breast tumor samples) is associated with PFS, separately in the fulvestrant only arm (i.e., the single agent arm) and the ganetespib plus fulvestrant arm (the combination arm). It is possible that the direction of the relationship between HSF1 and PFS is different in each arm, so we will test this hypothesis separately in the two arms. We hypothesize that high nuclear expression of HSF1 could be predictive of poor outcome in the fulvestrant-treated patients but could be predictive of improved outcome in patients treated with the combination of fulvestrant and ganetespib.

For the power calculations, we assume that the direction of effect could be in either direction for each of the treatment arms and we will use a two-sided test. We assume that all patients have tissue for analysis. Archival metastatic specimens will take priority over archival primary tumor, in situations when both are available. We will analyze the relationship of HSF1 (dichotomized as high positive versus low positive/negative) with a dichotomized variable for PFS (above/below median PFS calculated in each group separately). It is not known what the frequency of HSF1 high positivity is in patients with metastatic disease. However data from primary tumor specimens in the Nurses' Health Study indicate that 23% of ER+ tumors are HSF1 negative, 48% are HSF1 low, and 28% are HSF1 high. It is expected that the percent of patients with high nuclear

expression of HSF1 in metastatic specimens will be larger than the percent of primary patients with high nuclear HSF1 expression. It is also possible that patients who develop metastases are more likely to have high nuclear HSF1 expression in their archival primary tumor specimens than are patients who do not develop metastases. So it seems justified to assume that more than 28% of the patients on this study will have high nuclear HSF1 expression in their archival specimens. We assume that the percentage of patients entered on this study who have high nuclear HSF1 expression in archival specimens will be between 30% and 50%.

In these calculations, a two-sided significance level of 0.05 is used. The minimal acceptable power is assumed to be 80%. Because of the small sample sizes, sometimes a power between 81% and 83% was the smallest attainable power that was 80% or greater.

The primary analysis for the association of high nuclear expression of HSF1 and PFS will be based on a two-sided Fisher exact test of whether HSF1 high positivity is associated with a larger or smaller percent of patients with PFS greater than the median PFS on that arm. So, if the two arms of the study have different medians, then this comparison will be based on different medians in each group. This also means that in each arm (with the possible exception of the 11 out of the 71 total patients who may be lost to follow-up or begin non-protocol anti-cancer therapy before progression), at the time of analysis all patients will have had a PFS event before the group median or else be followed for PFS at least past the median PFS on that arm. (As a further secondary analysis, we will examine the time to PFS event for HSF1 high positive and HSF1 low positive/negative patients, separately for the two arms. However, we do not now have estimates of the distribution of time to PFS that are sufficiently robust for the two arms to allow us to do power calculations on this method of analysis.)

The power tables below show the range of differences that could be detected given the assumptions above.

For single-agent arm (fulvestrant alone):

			50% have high nuclear HSF1 expression			30% have high nuclear HSF1 expression			
Total N	Rando Ratio	This Arm N	Number HSF1 high pos	Detect. Diff.	True %s PFS > Med	Number HSF1 high pos	Detect. Diff.	True %s PFS > Med	True %s PFS > Med
60	1:2	20	10	68%	84%, 16%	6	67%	97%, 30%	70%, 3%

For combination arm (fulvestrant plus ganetespib**):**

			50% have high nuclear HSF1 expression			30% have high nuclear HSF1 expression			
Total N	Rando Ratio	This Arm N	Number HSF1 high pos	Detect. Diff.	True %s PFS > Med	Number HSF1 high pos	Detect. Diff.	True %s PFS > Med	True %s PFS > Med
60	1:2	40	20	50%	75%, 25%	12	52%	86%, 34%	66%, 14%

14.5.2 Comparisons of fulvestrant and ganetespib effects in cycle 2 day 9 biopsy tumor tissue

We will collect cycle 2 day 9 biopsies (required in patients with biopsy-accessible disease, see Section 3.1.14 and Section 8.1) to assess the effect of fulvestrant alone versus fulvestrant plus ganetespib within tumor specimens. This time point for the research biopsy was chosen to allow adequate time for fulvestrant to reach steady-state levels and to permit HSP90 client proteins modulating growth and survival to be destabilized by ganetespib, thereby inducing downstream transcriptional changes.

We assume that 20% of patients entered on study will have biopsy-inaccessible disease and that biopsies will not be done on these patients. We assume that 60% of patients who undergo biopsies will have informative tissue for analysis. Therefore, we assume that 48% (0.80 x 0.60) of patients accrued to the trial will have tissue available for this analysis.

We will compare the expression of ER, PR, AR, EGFR, HSF1, and HER2 by immunohistochemistry (IHC) and amplification of EGFR and HER2 using fluorescence in situ hybridization (FISH) to assess the effect of fulvestrant alone versus fulvestrant plus ganetespib within tumor specimens.

For this analysis, each of the markers will be described as "positive" or "negative". For ER, PR, AR, and EGFR, "positive" will be defined as $\geq$ 1% nuclear staining (which includes low positive and well as high positive) and "negative" will be defined as < 1% nuclear staining. For HSF1, "positive" will be defined as either 2+ staining or 1+ staining, and "negative" as no staining or rare cells positive (Santagata et al, manuscript in preparation). For HER2, we will use the standard 0-3+ scale, where categories 0, 1+, or 2+ count as negative and category 3+ counts as positive. For EGFR and HER2 amplification, FISH ratio $\geq$ 2.0 will be considered positive and FISH ratio < 2.0 will be considered negative. For each of these eight biologic markers, among patients who have usable tissue from the cycle 2 biopsy, we will test whether the fulvestrant arm or the fulvestrant plus ganetespib arm has a larger percent of patients with the positive category of the marker. This will be done with a two-sided Fisher exact test with significance level of 0.05. The minimal acceptable power was assumed to be 80%. Because of the small sample sizes, sometimes a power between 81% and 84% was the smallest attainable power that was 80% or greater. The power calculations assume that overall (both treatment arms combined), between 35% and 65% of patients would be in the positive category.

The power table below shows the types of differences that could be detected between the arms, given the assumptions above. While the tests are two-sided, because there are only half as many patients on the fulvestrant alone arm as are on the fulvestrant plus ganetespib arm, the differences that have power are slightly different depending on which arm is truly associated with a larger percent of patients with positive biomarker result.

48% of Patients with Usable Metastatic Tissue: True Treatment Difference in Percent of Patients with Positive Marker Detectable with *Two-Sided* Fisher Exact Test

			50% Specimens are Positive			35% are Positive		
N	N with Tissue	Arm with More Patients with "Positive" Marker	N with Positive Marker	Detect. Diff.	True % Positive Marker Combo, Single	N with Positive Marker	Detect Diff.	True % Positive Marker Combo, Single
60	28	Combo	14	58%	71%, 13%	10	54%	55%, 1%
60	28	Single	14	59%	29%, 88%	10	58%	15%, 73%

All of the other categorical correlative objectives based on the cycle 2 biopsy or (e.g., comparing the two arms by assessing a set of genes using a Nanostring custom code set each categorized as increased, decreased, or neutral) will also be compared using a two-sided Fisher exact test, but it isn't easy to describe the detectable difference for outcomes that have three categories rather than two categories of biomarker results. Fisher exact tests and descriptive statistics will be used to explore the relationship of biomarker results obtained from different types of specimens (cycle 2 biopsy, archival tissue, CTCs, and optional biopsies at the time of progression), both overall and within treatment arm. With only 28 patients treated on the two arms expected to have usable tissue from the cycle 2 biopsy, and with some of these patients expected to not have usable tissue from the other specimens sources, most of these comparisons will have very little power.

Exploring the relationship of PIK3CA mutations and other oncogenes and PFS in each arm separately may involve a few Kaplan-Meier curves and a few log rank tests, but with only 43 patients treated on both arms combined with a PFS event at the time of analysis, and with some of these patients not having usable biopsy tissue, this exploration may be based mostly on tables of estimated medians of time to PFS event.

14.6 Reporting and Exclusions

14.6.1 Evaluation of toxicity.

All participants will be evaluable for toxicity if they received any protocol therapy.

14.6.2 Evaluation of response.

All participants randomized on the study who have RECIST measurable disease must be assessed for response to treatment, even if there are major protocol treatment deviations, or they receive no treatment, or if they are ineligible. Participants who have no follow-up tumor evaluations, who die or receive non-protocol anticancer therapy before a response is noted, or who have unknown or not-assessable response will count as non-responders.

14.6.3 Evaluation of progression-free survival.

All participants randomized on the study must be assessed for PFS, even if there are major protocol treatment deviations, or they receive no treatment, or if they are ineligible. However, there will be no imputation of any missing progression-free survival times.

14.6.4 Analyses of Subsets of Patients.

For all endpoints except toxicity, the primary analysis will be based on all randomized patients (and the primary analysis of toxicity will be based on all patients who receive at least one dose of one of the study agents). However, in addition to the primary analysis, analysis of subsets of patients may be done (e.g., analysis restricted to eligible patients or to patients who receive at least one cycle of therapy). However, these analyses of subsets will be considered exploratory and will not serve as the basis for drawing conclusions about treatment efficacy.

15. PUBLICATION PLAN

The data will be collected and analyzed by Dr. Nancy Lin, in conjunction with a DFCI biostatistician, and key co-investigators. The results with be shared with Synta Pharmaceuticals. It is anticipated that results will be made public within 12 months of the end of data collection. Initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. The primary endpoint of the study will not be reported until the study is closed to accrual and the protocol-specified follow up period has elapsed. A report will also be published in a peer-reviewed journal. A full report of the outcomes will be made public no later than three years after the end of data collection.

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17. APPENDICES

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Appendix A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	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

Cross-sectional Interventional Radiology Service

Division of Abdominal Imaging and Intervention
Department of Radiology
Brigham and Women's Hospital

Experimental Protocol: DFCI 11-477: Fulvestrant +/- ganetespib for ER+ Breast Cancer

PI: Nancy Lin, MD DFCI pager # 42012

CIS MD Coordinator: Stuart Silverman, MD

Study Coordinators: Danielle Gore, Tel: 617-632-4870, DFCI Pager # 40503

Study Nurses: Kathleen Roche, Elizabeth Kasparian, Margaret Haldoupis, Mary O'Driscoll, Myra St. Amand, and Elizabeth Tiani. Main office tel: 617-632-3478.

Sites likely to be biopsied: abdomen, pelvis, lung, bone, lymph nodes, peritoneal fluid, pleural fluid

Synopsis: The goal of this clinical protocol is to collect tissue specimens from areas of loco-regional and distant metastases in patients with metastatic breast cancer being treated on a clinical trial of fulvestrant alone versus fulvestrant plus ganetespib. These specimens will ultimately be used to assess the effects of endocrine therapy alone versus endocrine therapy plus on Hsp90 inhibitor on a variety of DNA, RNA, and protein levels.

Coordination/Preparation:
Arranged through Percipio order entry system. The fact that it is a research biopsy must be indicated in Percipio along with the F number and the DFCI protocol number in Percipio. Study coordinator will specify in the Percipio order the biopsy time point. The order should specify the F number, and which organ is preferred, but should also state that the interventionalist has the discretion to choose a different site if thought to be safer or higher yield. If there is a question regarding the biopsy, the abdominal CSIR attending can be consulted before or after the order is placed.

DFCI personnel will be responsible for making sure that that patient has obtained a blue card (obtained via telephone 1-866-189-4056, that images are available for the CSIR team to review in advance of the Percipio request, arranging for appropriate blood work to be drawn (PT/INR, PTT, CBC), and notifying patients that 1) they should be expected to arrive 1 hr in advance of the biopsy, 2) the procedure will take approximately one hour, and 3) the recovery may last 4-6 hours or longer. Also they should be notified that someone will need to be available to drive them home. Additional instructions will be provided by the CSIR nursing staff. For questions, call CSIR coordinators at 617-732-7785. DFCI team will be present for procedure and to transport tissue, provide materials, fixatives, and requisitions. The DFCI study coordinator will receive and transport research specimens to the appropriate labs.

Special Needs: Off Coumadin x 5 days and then recheck labs. Patients should be off ASA for at least 5 days, or heparin (e.g., fragmin, Arixtra (LMW Heparin compound)) or NSAIDs for more than 48 hours. If there are risks of stopping these medications, these should be discussed with other care providers by DFCI personnel before the referral to CSIR.

Billing: Funding is provided by a Komen grant. F # 8151631. Contact for billing: Thomas Deveau or James Huse, Clinical Trials Business Office, Ph 617-582-8406 or 617-632-6144, DFCI BP332A.

Note: Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

Procedure (if not bone):

Set	Needle	# Sample	Preparation	Destination
1	18-20G cores*	3-6	Dry/cup	DFCI SM932 DFCI pager# 49233 for pick up/processing.

*use 18G if safe to do so. Can use 20G or obtain fewer specimens, at discretion of interventionalist for safety reasons

*if for safety reason only 22G fine needle aspiration biopsies are thought possible (whether per treating physician or interventionalist), then use the following protocol:

Set	Needle	# Sample	Preparation	Destination
1	FNAB	3*	*	DFCI SM932 DFCI pager# 49233 for pick up/processing.

*Special notes regarding sample collection/preparation:

1. One pass should be evacuated and rinsed directly into 2mL of room temperature Trizol for RNA analysis.
2. One pass should be evacuated and rinsed directly into 2mL of room temperature Trizol for DNA or RNA analysis.

3. One pass should be evacuated and rinsed directly into 10-20mL of RMPI to prepare a cell block

Procedure (bone ONLY):

Set	Needle	# Sample	Preparation	Destination
1	11-13G	1-3	Dry/cup	DFCI SM932 DFCI pager# 49233 for pick up/processing.

Procedure (Paracentesis or Thoracentesis)

Set	Needle	# Sample	Preparation	Destination
1	*	500cc	Standard collection tube/bottle	DFCI SM932 DFCI pager# 49233 for pick-up/processing

*As clinically indicated.

SGS rev 6-18-12

APPENDIX C:
Tissue and Blood Acquisition Guidelines

FOR DANA FARBER CANCER INSTITUE AND BRIGHAM& WOMEN'S HOSPITAL LAB USE ONLY

For Biopsies of Soft Tissue, Liver, Bone, Breast, Etc.

1. After biopsy is performed, the tissue mass is placed on a sterile gauze
2. Using forceps, separate the tumor tissue
3. Place 2 pieces (cores) of tumor tissue on a pre-frozen bed of OCT in each cassette (typically end up with 3 cassettes per biopsy); the last cassette will contain many small pieces of tumor tissue
4. Fill cassettes with OCT
    a. Completely cover tissue with additional OCT
    b. Limit the amount of bubbles
5. Tissue must be frozen immediately by placing cassettes on dry ice in a biohazard cooler
6. Transport samples to the lab (Smith building, 9th floor, Room SM-948) and complete freezing of samples in OCT with dry ice (about 10 minutes freezing time)
7. Once samples are frozen, place in plastic bag; label bag with date, protocol number, patient number, and number of initials included
8. Store in –80C freezer

For Effusions and Ascites

Email notification should be sent to Danielle Gore at dgore@partners.org to arrange sample pick up.

Samples are to be collected in a sterile vacutainer.

The coordinator will pick up ascites/ pleural fluid from BWH Cytology for cell pellet processing in Dr. Richardson's lab (Smith building, 9th floor, Room SM-948)
Fluid sample should be split into three equal aliquots.

One aliquot should be spun down into a pellet and snap frozen in an ETOH/dry ice bath or in liquid N2. This sample is stored in Dr. Richardson's lab and should be registered in caTissue Blood Tissue Bank.

One aliquot should be spun down into a pellet and fixed by Dr. Richardson's lab,, and processed as a standard cell block by the Specialized Histopathology Services at BWH (Thorn building, 6th floor, Rooms 604/603B) . The request for a standard cell block is submitted online through the DF/HCC Research Pathology Cores website: http://genepath.med.harvard.edu:8080/pathcore/ and it should specify: Specialized Histopathology Services (SHL): Cell paraffin block preparation from ascites/pleural fluid. Then, the fixed sample is taken to the Specialized Histopathology Services at BWH for processing as a standard block along with a print out of request, (Note: if the sample preparation is done by a clinical cytopathology laboratory, it is important to explain that the sample is for research purposes only and that no thin prep should be performed as this uses up a significant portion of the sample.)

One aliquot should be spun down into a pellet that should be washed and re-suspended in PBS, and then transferred into Cell Save preservative tubes (7.5 mL) in the research lab. Samples should be submitted together with a Circulating Tumor Cell Core Facility request form to BWH CTC Core Facility.

Pleural fluid samples can be transported and stored at room temperature (15-30 ^{o}C) up to 72 hours before processing. Do not refrigerate or freeze the sample. Samples must be processed within 72 hours of collection, but best results are obtained if the sample is processed as soon as possible.

For Fine Needle Aspiration Samples

For patients who undergo fine needle aspiration (FNA), 3 passes should be collected:

The first pass should be evacuated and rinsed directly into 2mL of room temperature Trizol to collect the RNA; a second pass should be evacuated and rinsed directly into another 2mL of room temperature Trizol to collect the DNA; the last pass should be evacuated and rinsed directly into 10-20mL of RPMI and prepared as a cell block.

The 2 samples suspended in trizol are to be brought to April Greene-Colozzi in Dr. Richardson's lab (Smith 486) for RNA & DNA analysis. The 1 aliquot in RPMI should be spun down into a cell pellet, washed and resuspended in ethanol (by Richardson lab), and brought to Cytopathology:

Brigham and Women's Hospital
Medical Research Building, Room 306
c/o Joyce Bookbinder
617-732-4715

Appendix H should accompany the sample. Sample should be labeled with a BOT number (assigned by Richardson lab; must be requested from April).

Joyce's team will create the block and send it to Histology to embed in paraffin. When complete (appx 24 hours), the block is ready for pickup.

Block is picked up Amory 3 Histology in BWH.

For CTC Samples

Blood samples are to be collected at at the timepoints specified in the Specimen Submission Calendar (Section 9.5). . Samples may be drawn Monday through Friday but not the day before a holiday (unless prior arrangements to receive the sample have been made).

1. Samples are to be collected in Cell Save preservative tubes (7.5 mL). Label tube with sample identifier / study ID number, protocol number, time point, and date of collection.
2. Draw a minimum of 5 mL of blood/tube (required in order to process the sample).
3. Gently invert each tube 4 times to ensure proper mixing of the blood with the fixative.
4. Blood samples can be shipped/transported and stored at room temperature (15-30 ^{o}C) up to 72 hours before processing. Do not refrigerate or freeze the sample. Samples must be processed within 72 hours of collection, but best results are obtained if the sample is processed as soon as possible. Samples should be submitted with a CTC request form. Do not submit clotted samples or samples containing less than 4ml of blood. Samples should be shipped by same day courier or overnight parcel directly to:

Dana-Farber Cancer Institute
Attn: Danielle Gore
450 Brookline Ave., DA157
Boston, MA 02215

Email notification should be sent to dgore@partners.org prior to shipment of sample. Danielle will send a reply email to confirm receipt of the sample.

Blood for Germline DNA

One 10 mL lavender top tube will be collected at baseline and banked in order to extract germline DNA to be used as normal DNA reference for tumor tissue-based studies.

Blood for cfDNA:

One 10 mL Streck tube will be collected at the timepoints specified in Section 9.5, Specimen Submission Calendar. The tube must be filled completed and gently inverted 8-10 times immediately after collection. Blood collected in the Streck tube can be stored for 14 days between 6-37 degrees C and can be mailed at room temperature.

Blood for PBMC samples

Blood will be drawn into two PAXgene blood RNA tubes (ref #762165) from Qiagen/BD. After the blood draw, samples will sit at room temperature for two hours and then sit for 24 hours in a -20º C freezer. Following this, samples will be stored at -80º C until several samples have accumulated. Blood samples are to be collected at the timepoints specified in Section 9.5, Specimen Submission Calendar. Samples will be stored at -80º C until pick-up is coordinated with a member of Dr. Whitesell's laboratory at Whitehead Institute for Biomedical Research in Cambridge, MA.

BRIGHAM AND WOMEN'S HOSPITAL

CIRCULATING TUMOR CELL CORE LABORATORY - REQUISITION FORM

Alarice Lowe, M.D., Director
75 Francis St., MRB-315
Boston, Massachusetts 02115
Tel: 617-525-8696 Fax: 617-739-6192 aclowe@partners.org

REQUIRED CLIENT INFORMATION:

Principal Investigator_____
Additional Contact_____
Results via mail, fax, or both: _____
Address_____
_____
Fax_____
Email_____
Telephone_____

Billing Information :

Partners Institutions:
PeopleSoft Business Unit:_____
Fund #_____
Partners Fund end Date:_____

All Others (including DFCI):
Email Invoice to:_____

Tube	Sample ID #	Protocol #	Draw Date	Draw Time	Service Requested (check one)*
1		**11-477**			☐ Enumeration ☒ Profile Kit (select one) ☐ cytospin - methanol fixation ☐ cytospin - 3:1 fixation ☒ cytospin - air dried ☐ tube
2		**11-477**			☐ Enumeration ☒ Profile Kit (select one) ☐ cytospin - methanol fixation ☐ cytospin - 3:1 fixation ☒ cytospin - air dried ☐ tube
3		**11-477**			☐ Enumeration ☒ Profile Kit (select one) ☐ cytospin - methanol fixation ☐ cytospin - 3:1 fixation ☒ cytospin - air dried ☐ tube

* Enumeration uses an Epithelial Cell Kit and provides a numerical result.
* Profile Kit isolates cells for downstream analysis.

For Lab Use Only:

Date and time processed
Initials/Comments

Appendix D

Tissue Specimen Release Form

[Date]

Dear «FName» «MName» «LName»:

Thank you very much for your participation in "DFCI 11-477: Randomized Phase II Trial of Fulvestrant versus Fulvestrant + Ganetespib for Hormone Receptor-Positive Breast Cancer." In order to study the biology of breast cancer, we need to examine actual tumor specimens potentially evaluating molecular and genetic markers that may help us further understand why some tumors respond to certain treatments but others do not. The research sample we have collected from your biopsy, coupled with samples of existing stored tumor specimens from pathology departments will make potential biological studies uniquely valuable.

We are therefore attempting to obtain existing tissue blocks and slides available from previous procedures (such as biopsies or surgeries). We hope you will agree to help in this research effort by reading the attached HIPAA notice and signing the specimen release form below. We will then contact the institution(s) where you had your surgery (-ies).

Thank you very much for your cooperation. If you should have any questions about this study, please do not hesitate to contact Clinical Research Coordinator, TBD at DFCI at <add phone, add email>.

Sincerely,

Nancy Lin, MD
Principal Investigator

I have read the attached HIPAA information regarding my protected health information and hereby grant permission to the research team at the Dana Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215 to examine the pathology records and specimens pertaining to my diagnosis of breast cancer.

Signed:_____ Date:_____

Name:_____Birthdate:_____

Where Initial Breast Surgery was done (If applicable)_____

Address:_____

City:_____State:_____Zip:_____

Where Biopsy (ies) of Recurrence was done (If applicable)_____

_

Address:_____

City:_____State:_____Zip:_____

Appendix D

Tissue Specimen Release Form

<Insert DF/HCC institution address here>

Subject Name

Date of Birth SSN

I hereby grant the <insert DF/HCC institution here> permission to obtain my tissue specimens from outside institutions.

Signature of Subject Date

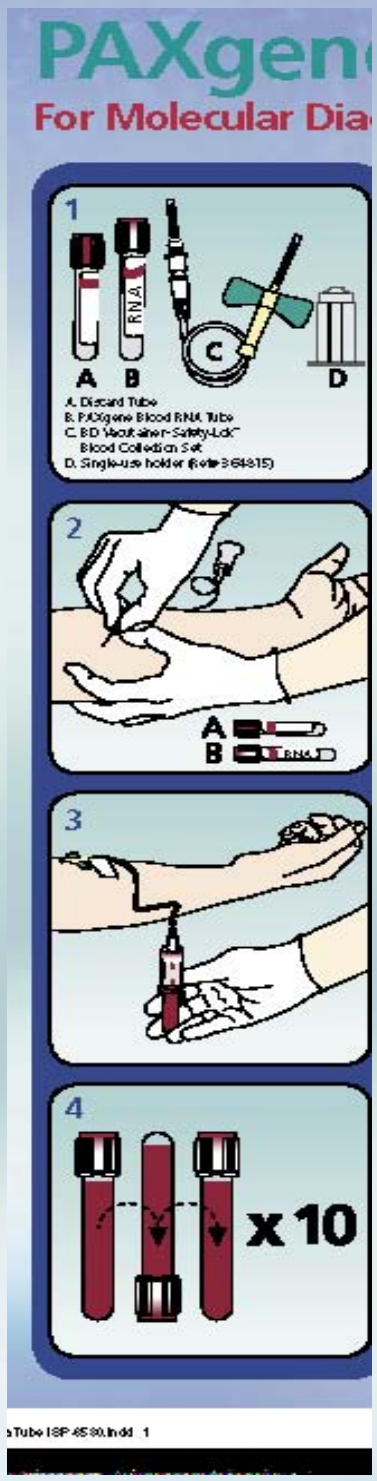
Please send tissue specimen(s) to:

<insert contact information here>

How to Collect Blood Using the

PAXgene™ Blood RNA Tube

For Molecular Diagnostic Testing



1

2

3

4

Required Items:

1a. Ensure that the PAXgene Blood RNA Tube (B) is at room temperature (18°C-25°C) prior to use and properly labeled with patient identification.

1b. If the PAXgene Blood RNA Tube is the only tube to be drawn, a small amount of blood should be drawn into a "Discard Tube" (A) prior to drawing blood into the PAXgene Blood RNA Tube. Otherwise, the PAXgene Blood RNA Tube should be the last tube drawn in the phlebotomy procedure.

RNA Stabilization Reagent

RNA StabilizedWhole Blood

Ref# 762165

Venipuncture:

2. Using a BD Vacutainer® Safety-Lok™ Blood Collection Set (C), collect blood into the PAXgene Blood RNA Tube using your institution's recommended standard procedure for venipuncture.

BD Vacutainer® Safety-Lok™ Blood Collection Set
Ref# 367281 North America
Ref# 367286 Other Countries

Blood Collection:

3a. Hold the PAXgene Blood RNA Tube vertically, below the blood donor's arm, during blood collection.

3b. Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. (See Figure 1)

After Blood Collection:

4a. Gently invert the PAXgene Blood RNA Tube 8 to 10 times.

4b. Store the PAXgene Blood RNA Tube upright at room temperature (18°C-25ºC) or at 4ºC.*

BD Customer Service/Orders:
888.237.2762 (North America)
32.53.720.337 (Europe)

PreAnalytiX
A QIAGEN / BD Company

www.PreAnalytiX.com

*Refer to PAXgene® Blood RNA Tube handbook.

Cell-Free DNA BCT®

Streck

INSTRUCTIONS FOR USE

INTENDED USE

Cell-Free DNA BCT® is a direct draw whole blood collection tube intended for collection, stabilization and transportation of cell-free plasma DNA. This device also stabilizes and preserves cellular genomic DNA present in nucleated blood cells and circulating epithelial cells (tumor cells) found in whole blood. **This product has not been cleared by the U.S. Food and Drug Administration for In Vitro Diagnostic use. The product is For Research Use Only. Not for use in diagnostic procedures.**

SUMMARY AND PRINCIPLES

Accurate analysis of cf-DNA can be compromised by sample handling, shipping and processing, causing lysis of nucleated blood cells and subsequent release of cellular genomic DNA. Additionally, degradation of cf-DNA due to nuclease activity can be problematic.

The formaldehyde-free preservative reagent contained in Cell-Free DNA BCT[1,2] stabilizes nucleated blood cells, preventing the release of cellular genomic DNA, and inhibits nuclease mediated degradation of cf-DNA, contributing to the overall stabilization of cf-DNA[3]. Samples collected in Cell-Free DNA BCT are stable for up to 14 days at temperatures between 6-37°C, allowing convenient sample collection, transport and storage[4].

The formaldehyde-free preservative reagent contained in Cell-Free DNA BCT stabilizes circulating epithelial cells (tumor cells) in whole blood for up to 4 days at temperatures between 15-30°C[5].

REAGENTS

Cell-Free DNA BCT contains the anticoagulant K_3EDTA and a cell preservative in a liquid medium.

PRECAUTIONS

1. **For Research Use Only. Not for use in diagnostic procedures.**
2. Do not freeze specimens collected in Cell-Free DNA BCT as breakage could result.
3. Do not use tubes after expiration date.
4. Do not use tubes for collection of materials to be injected into patients.
5. Product is intended for use as supplied. Do not dilute or add other components to Cell-Free DNA BCT.
6. Overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytic results or poor product performance.
7. CAUTION
   a. Glass has the potential for breakage; precautionary measures should be taken during handling.
   b. All biological specimens and materials coming in contact with them are considered biohazards and should be treated as if capable of transmitting infection. Dispose of in accordance with federal, state and local regulations. Avoid contact with skin and mucous membranes.
   c. Product should be disposed with infectious medical waste.
   d. Remove and reinsert stopper by either gently rocking the stopper from side to side or by grasping with a simultaneous twisting and pulling action. A "thumb roll" procedure for stopper removal is NOT recommended as tube breakage and injury may result.
8. SDS can be obtained at www.streck.com or by calling 800-843-0912.

STORAGE AND STABILITY

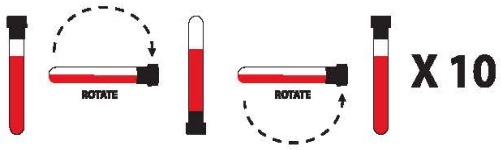
1. When stored at 18-30°C, unused Cell-Free DNA BCT is stable through expiration date.
2. Do not freeze unfilled Cell-Free DNA BCT. Proper insulation may be required for shipment during extreme temperature conditions.
3. Blood samples collected in Cell-Free DNA BCT for cf-DNA analysis are stable for 14 days when stored between 6-37°C.
4. Blood samples collected in Cell-Free DNA BCT for genomic DNA analysis are stable for 14 days when stored between 6-37°C.
5. Blood samples collected in Cell-Free DNA BCT for circulating epithelial cells (tumor cells) are stable for 4 days when stored between 15°-30°C.

INDICATIONS OF PRODUCT DETERIORATION

1. Cloudiness or precipitate visible in reagent of unused tube.
2. If indications of product deterioration occur, contact Streck Technical Services at 800-843-0912 or technicalservices@streck.com.

INSTRUCTIONS FOR USE

1. Collect specimen by venipuncture according to CLSI H3-A6[6].
   Prevention of Backflow - Since Cell-Free DNA BCT contains chemical additives, it is important to avoid possible backflow from the tube.
   To guard against backflow, observe the following precautions:
   a. Keep patient's arm in the downward position during the collection procedure.
   b. Hold the tube with the stopper in the uppermost position so that the tube contents do not touch the stopper or the end of the needle during sample collection.
   c. Release tourniquet once blood starts to flow in the tube, or within 2 minutes of application.
2. Follow recommendations for order of draw outlined in CLSI H3-A6[6].
3. Fill tube completely.
4. Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate test results. One inversion is a complete turn of the wrist, 180 degrees, and back per the figure below:



5. After collection, transport and store tubes within the recommended temperature range.
6. Perform extraction in accordance with instrument manufacturer's instructions. For optimal results, please follow the directions for cell-free plasma DNA and cellular genomic DNA extraction.

CELL-FREE PLASMA DNA AND CELLULAR GENOMIC DNA EXTRACTION

1. Extraction of cell-free plasma DNA and cellular genomic DNA can be accomplished using most commercially available kits.
2. For optimal results, include a Proteinase K treatment step (≥ 30 mAU/ml digest) at 60°C in the presence of chaotropic salts for 1 hour when extracting cell-free DNA and for 2 hours when extracting cellular genomic DNA.

Note:

a. Cell-Free DNA BCT does not dilute blood samples; therefore, no dilution factor correction is necessary to obtain absolute count values.
b. As in the case with most clinical laboratory specimens, hemolysis, icterus and lipemia may affect the results obtained on blood samples preserved with Cell-Free DNA BCT.

LIMITATIONS

1. Unused tubes to be stored between 18-30°C.
2. Samples drawn in other anticoagulants or preservatives may cause coagulation in Cell-Free DNA BCT.

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ORDERING INFORMATION

Please call our Customer Service Department toll free 800-228-6090 for assistance. Additional information can be found online at www.streck.com.

GLOSSARY OF HARMONIZED SYMBOLS

EC REP Authorized Representative in the European Community	LOT Batch Code	Biological Risk	REF Catalog Number	Use By
IVD In Vitro Diagnostic Medical Device	Manufacturer	Consult Instructions For Use	Temperature Limitation	Do Not Re-use

Glossary of symbols may contain symbols not used in the labeling of this product.

See www.streck.com/patents for patents that may be applicable to this product.

Streck
7002 S. 109 Street Omaha, NE 68128 USA

350547-6
2014-04

DFCI IRB Protocol #: 11-477

APPENDIX G

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

TABLE OF CONTENTS

1.0 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), Children's Hospital Boston (CHB), 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 (CTEP, Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) 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. CTEP Protocol and Information Office (PIO), FDA, OBA etc.). 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 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 (i.e. CTEP Multi-Center 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 unit within DF/HCC developed to computerize and manage data, and to provide a Quality Control and Quality Assurance function for DF/HCC trials.

2.0 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, Dr. Nancy Lin, 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.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team 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 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 (investigator-held IND trials) as applicable.
- 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.

2.2 Coordinating Center

The Coordinating Center will assume the following general responsibilities:

- Assist in protocol development
- Maintain copies of Federal Wide Assurance and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain FDA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to DF/HCC Sponsor for timely review.
- Distribute adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all participating investigators.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Monitor Participating Institutions either by on-site or virtual monitoring.
- Maintain Regulatory documents of all Participating Institutions.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc).
- Maintain documentation of all communications.
- Ensure that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP).

2.3 DF/HCC Quality Assurance Office for Clinical Trials (QACT)

In addition to the Coordinating Center, the DF/HCC QACT provides the following support services to assist the DF/HCC Sponsor:

- Develop protocol specific case report forms (eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide a central participant registration, which includes review of consent and eligibility.
- Provide auditing services (funding and QACT approval required).

2.4 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements. All Participating Institutions will provide a list of personnel assigned to the role for oversight of data management at their site to the Coordinating Center.

The general responsibilities for each Participating Institution are as follows:

- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder in accordance with DF/HCC requirements.

- Provide the Coordinating Center with regulatory documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- 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.
- Secure and store 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.
- For protocols using investigational agents, the Participating Institution will order their own investigational agents regardless of the supplier (i.e. pharmaceutical company).

3.0 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 90 days from receipt of the notification.

- **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.

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:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution’s IRB
- Participating IRB’s approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the Coordinating Center their IRB approval for amendments to a protocol.

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 (HIPAA). 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. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB, will provide a consent template, which covered entities (Participating Institutions) must use.

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 per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.7 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 must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number (as described below) and DF/HCC protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification.

3.8 DF/HCC Multi-Center Protocol Registration Randomization

Please refer to Protocol Section 4.3 for participant registration and randomization information. Treatment cannot begin until site has received confirmation that the participant has been registered with DFCI QACT.

3.9 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 a faxed or e-mailed copy of the participant's registration confirmation memo from the Coordinating Center. Therapy must be initiated per protocol guidelines. The DF/HCC Sponsor and DFCI IRB must be notified of any exceptions to this policy.

3.10 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.

3.11 Verification of Registration, Dose Levels, and Arm Designation

A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one business day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

3.12 DF/HCC Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and correspondence, and correspondence with the Coordinating Center.

3.13 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 derivations 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.

3.13.1 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.

3.13.2 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 to CTEP via an internal DF/HCC process, as applicable.

4.0 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 NCI 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.

4.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 11.

Participating Institutions must report the AEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

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 Investigators will review any distributed AE reports, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

4.2 Guidelines for Processing IND Safety Reports

FDA regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any adverse experience associated with the use of the investigational agent that is both serious and unexpected. The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Institutions will review, and submit to their IRB according to their institutional policies and procedures.

5.0 DATA MANAGEMENT

The DF/HCC QACT develops a set of either paper or electronic 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.

5.1 Data Forms Review

When data forms arrive at the DF/HCC QACT, they are reviewed for completeness, protocol treatment compliance, adverse events (toxicities) and response. Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

5.2 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 or study monitor. 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.

5.3 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 a minimum of four times a year.

6.0 REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol.

Participating Institutions should order their own agent regardless of the supplier (i.e., a pharmaceutical company.)

If the agent is commercially available, check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB.

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

7.0 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.

7.1 Ongoing Monitoring of Protocol Compliance

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

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur before the clinical phase of the protocol begins, continue during protocol performance and through study completion. Additional 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 files, protocol deviations, pharmacy records, response assessments, and data management.

Participating institutions will be required to participate in monthly Coordinating Center initiated teleconferences. "Newsletters" highlighting overall protocol progress and important announcements will be distributed as deemed appropriate by the protocol chair.

Virtual monitoring visits will begin after at least 3 participants have been enrolled or after 1 participant has been enrolled if no additional enrollment has occurred in a 6 month period. Virtual monitoring will continue approximately every 6 months and may occur more frequently if there are significant findings or discrepancies. Virtual monitoring will be performed by the Coordinating Center's Clinical Research Specialist (CRS). The data will be reviewed for completeness, quality, and adherence to the protocol requirements. Sites will be asked to provide de-identified source documentation via fax, email, or mail as specified by the Clinical Research Specialist for all virtual monitoring visits. A virtual site initiation visit (SIV) will be conducted with each participating site prior to study activation. Participating sites many not begin enrolling until the SIV has occurred.

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Coordinating Center and if applicable, QACT Data Analysts assigned to the protocol, will perform the ongoing protocol data compliance monitoring.

7.2 Evaluation of Participating Institution Performance

7.2.1 Monitoring Reports

The DF/HCC Sponsor will review all Participating site monitoring reports to ensure protocol compliance and to fulfill sponsor responsibilities. 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.

8.0 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).

8.1 DF/HCC Sponsored Trials

One on-site audit will be conducted by QACT assuming at least 3 participants are treated at a participating site. An audit may be triggered if deficiencies are noted related to consent practices, eligibility, missing, incomplete, or questionable data submission, or any other issue the protocol chair deems appropriate for audit. If a participating site is selected for audit, approximately 3-4 participants would 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.

8.2 Participating Institution

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal) 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.

8.3 DF/HCC Sponsor and Coordinating Center

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.

8.4 Sub-Standard Performance

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

8.5 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will 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.

APPENDIX H: HISTOLOGY LABORATORY PROJECT REQUEST FORM

DEPARTMENT OF PATHOLOGY

HISTOLOGY LABORATORY PROJECT REQUEST FORM

ACCESSION NUMBER:

BR _____- _____

Teaching:

Project:

Researcher Name: _PI: Nancy Lin, MD (CRC: Danielle Gore)
Date Submitted: MM/DD/YY
Researcher Contact Information: nlin@partners.org (617 632 6973;
dgore@partners.org (617-632-4174)
DFCI Protocol #: 11-477

Grant#/Cost Center #/Purchase Order#: F8151631
IMPORANT NOTE: This must be a current PeopleSoft Number for you to charge to the grant directly to Kronos. If you are not sure, you must check with your requestor.

BILLING INFORMATION

Contact Name & INSTITUTION: Jennifer Savoie

Contact Address: Dana-Farber Cancer Institute 450 Brookline Avenue, Yawkey 1250, Boston MA 02215

Contact Phone Number/Email Address: dgore@partners.org 617 632 4870

Estimated Cost of Supplies: _____
(Prior authorization required by manager for cost estimate)

Researcher: Please fill out the detailed description of type of work performed (Include number and type of slides and stains, etc.)

GENERAL/SPECIAL INSTRUCTION:

Need a cell block pellet to be processed and embedded and 1 H&E section cut from block. Label as **BOT XXXX** and place in Dr. Andrea Richardson's mailbox in pathology.

Number of Blocks to be processed/embedded: 1 **Number of special stains:**

Number of H&E slides: 1 **Number of IHC/ISH:**

Number of unstained/no bake slides: **Other:**

	Sunday Date: / /	Monday Date: / /	Tuesday Date: / /	Wednesday Date: / /	Thursday Date: / /	Friday Date: / /	Saturday Date: / /
Time In ********** Time Out ********** Total Hours Worked	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____
TECHNICAL DIRECTOR TIME ESTIMATE		TECHNICIAN ASSIGNED TO PROJECT:					

PROJECT APPROVED BY (Jim Pepoon or Jack McCabe):

_____

PLEASENOTE: ALL OVERTIME REQUESTS MUST BE DISCUSSED WITH THE LAB MANAGER BEFORE PROJECTS CAN BEGIN. TECHNICIANS WILL BE ASSIGNED/APPROVED BASED ON LABORATORY WORKLOAD. TIME REPORT MUST BE COMPLETED EACH WEEK AND SUBMITTED WITH TECHNICIAN'S WEEKLY TIMESHEET.

APPENDIX I: List of Drugs Known to Predispose to Torsades de Pointes

Substantial evidence supports the conclusion that these drugs, when used as directed in labeling, can prolong the QT interval and can have a risk of Torsades de pointes (TdP) in some patients.

Generic Name	Brand Name	Class/Clinical Use	Comments
Amiodarone	Cordarone®	Anti-arrhythmic / abnormal heart rhythm	Females>Males,TdP risk regarded as low
Amiodarone	Pacerone®	Anti-arrhythmic / abnormal heart rhythm	Females>Males,TdP risk regarded as low
Arsenic trioxide	Trisenox®	Anti-cancer / Leukemia	
Astemizole	Hismanal®	Antihistamine / Allergic rhinitis	No Longer available in U.S.
Azithromycin	Zithromax®	Antibiotic / bacterial infection	
Bepridil	Vascor®	Anti-anginal / heart pain	Females>Males
Chloroquine	Aralen®	Anti-malarial / malaria infection	
Chlorpromazine	Thorazine®	Anti-psychotic/ Anti-emetic / schizophrenia/ nausea	
Cisapride	Propulsid®	GI stimulant / heartburn	No longer available in U.S.
Citalopram	Celexa®	Anti-depressant / depression	
Clarithromycin	Biaxin®	Antibiotic / bacterial infection	
Disopyramide	Norpace®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Dofetilide	Tikosyn®	Anti-arrhythmic / abnormal heart rhythm	Females > Males
Domperidone	Motilium®	Anti-nausea / nausea	Not available in U.S.
Dronedarone	Multaq®	Anti-arrhythmic / Atrial Fibrillation	
Droperidol	Inapsine®	Sedative;Anti-nausea / anesthesia adjunct, nausea	
Erythromycin	E.E.S.®	Antibiotic;GI stimulant / bacterial infection; increase GI motility	Females>Males
Erythromycin	Erythrocin®	Antibiotic;GI stimulant / bacterial infection; increase GI motility	Females>Males
Escitalopram	Cipralex®	Anti-depressant / Major depression/ Anxiety disorders	Changed from Possible Risk to Risk- 12/2/12
Escitalopram	Lexapro®	Anti-depressant / Major depression/ Anxiety disorders	Changed from Possible Risk to Risk- 12/2/12
Flecainide	Tambocor®	Anti-arrhythmic / abnormal heart rhythm	
Halofantrine	Halfan®	Anti-malarial / malaria infection	Females>Males
Haloperidol	Haldol®	Anti-psychotic / schizophrenia, agitation	TdP risk with I.V. or excess dosage
Ibutilide	Corvert®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Levomethadyl	Orlaam®	Opiate agonist / pain control, narcotic dependence	Not available in U.S.
Mesoridazine	Serentil®	Anti-psychotic / schizophrenia	Removed from US market in 2004
Methadone	Dolophine®	Opiate agonist / pain control, narcotic dependence	Females>Males
Methadone	Methadose®	Opiate agonist / pain control, narcotic dependence	Females>Males
Moxifloxacin	Avelox®	Antibiotic / bacterial infection	
Ondansetron	Zofran®	Somatostatin analog / nausea and vomiting	
Ondansetron	Anset®	Somatostatin analog / nausea and vomiting	
Ondansetron	Zuplenz®	Somatostatin analog / nausea and vomiting	
Ondansetron	Emetron®	Somatostatin analog / nausea and vomiting	
Ondansetron	Ondavall®	Somatostatin analog / nausea and vomiting	

Generic Name	Brand Name	Class/Clinical Use	Comments
Pentamidine	NebuPent®	Anti-infective / pneumocystis pneumonia	Females>Males
Pentamidine	Pentam®	Anti-infective / pneumocystis pneumonia	Females>Males
Pimozide	Orap®	Anti-psychotic / Tourette's tics	Females>Males
Probucol	Lorelco®	Antilipemic / Hypercholesterolemia	No longer available in U.S.
Procainamide	Pronestyl®	Anti-arrhythmic / abnormal heart rhythm	
Procainamide	Procan®	Anti-arrhythmic / abnormal heart rhythm	
Quinidine	Quinaglute®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Quinidine	Cardioquin®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Sevoflurane	Ulane®	Anesthetic, general / anesthesia	Label warning for patients with congenital long QT or patients taking QT prolonging drugs
Sevoflurane	Sojourn®	Anesthetic, general / anesthesia	Label warning for patients with congenital long QT or patients taking QT prolonging drugs
Sotalol	Betapace®	Anti-arrhythmic / abnormal heart rhythm	Females>Males
Sparfloxacin	Zagam®	Antibiotic / bacterial infection	No longer available in U.S.
Terfenadine	Seldane®	Antihistamine / Allergic rhinitis	No longer available in U.S.
Thioridazine	Mellaril®	Anti-psychotic / schizophrenia	
Vandetanib	Caprelsa®	Anti-cancer / Thyroid cancer	

APPENDIX J: 11-477 SPECIMEN REQUISITION (Archival Tissue Blocks/Slides and CTC Samples)

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

Ship specimen(s) to: Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Specimen Information

Participant Initials (FML): _____ DFCI Participant Study ID Number: _____ Date specimen(s) shipped:_____

Time Point ☐ Pre-study/Baseline ☐ Cycle 1 Day 1 ☐ Cycle 2 Day 8/Day 9 ☐ Off Treatment (without progression)
☐ Progression on 1st Regimen (Arm A or B) ☐ Progression on 2nd Regimen (Arm C) ☐ Off Study

Specimen Type *(indicate inclusion in shipment by checking box)*	**Pathology Number(s) or Serial Coding**	**Quantity submitted**	**Date specimen obtained**	**Time specimen obtained** (24 Hour Clock)
☐ Archival tissue block				
☐ 10-20 unstained slides				
☐ CTC (CellSave) tubes				
☐ Other, specify:				
☐ Other, specify:				

Responsible contact: _____

Email: _____

Phone number: _____

Site: _____

Mailing address (if blocks need to be returned): _____

_____

_____

_____

APPENDIX K: 11-477 SPECIMEN REQUISITION (Biopsy/Research Blood)

Complete this form and include with the specimen shipment. Label ALL materials with participant initials, DFCI participant study ID, collection time point and collection date.

Ship specimen(s) to: Danielle Gore
Dana-Farber Cancer Institute
450 Brookline Avenue, DA157
Boston, MA 02215

Specimen Information

Participant Initials (FML): _____ DFCI Participant Study ID Number: _____ Date specimen(s) shipped: _____

Time Point ☐ Pre-study/Baseline ☐ Cycle 1 Day 1 ☐ Cycle 2 Day 8/Day 9 ☐ Off Treatment (without progression)
☐ Progression on 1st Regimen (Arm A or B) ☐ Progression on 2nd Regimen (Arm C) ☐ Off Study

Specimen Type *(indicate inclusion in shipment by checking box)*	**Quantity submitted**	**Date specimen obtained**
☐ Biopsy core(s) frozen in OCT		
☐ FNA samples		
☐ Fluid biopsy samples		
☐ Streck tubes		
☐ Blood in lavender top (EDTA)		
☐ Other, specify:		
☐ Other, specify:		

Responsible Contact: _____ **Email:** _____

Site: _____ **Phone number:** _____

APPENDIX L: 11-477 SPECIMEN REQUISITION (PBMC Tubes)

Complete this form and include with the specimen shipment. Label ALL materials with DFCI participant study ID, collection time point and collection date and time.

Ship specimen(s) to: Luke Whitesell
Whitehead Institute
9 Cambridge Center
Cambridge, MA 02142

Specimen Information

DFCI Participant Study ID Number: _____ Date specimen(s) shipped: _____

PBMC (PAXgene) Tube Time Point *(indicate inclusion in shipment by checking box)*	**Quantity submitted**	**Collection Date**	**Collection Time** (24 Hour Clock)
☐ Pre-treatment/Cycle 1 Day 1			
☐ Cycle 2 Day 8			
☐ Cycle 2 Day 9			
☐ Progression on 1st Regimen (Arm A or B)			
☐ Progression on 2nd Regimen (Arm C)			
☐ Off Treatment (without progression)			
☐ Off Study			

Responsible Contact: _____ **Email:** _____

Site: _____ **Phone number:** _____

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**11-477**
Protocol Name:	**Randomized phase II study of fulvestrant with or without ganetespib in patients with hormone receptor-positive, metastatic breast cancer**
DFCI Site PI:	Nancy Lin, MD
DFCI Research Nurse:	Myra St. Amand, RN; Kathleen Roche, RN; Elizabeth Kasparian, RN; Margaret Haldoupis, RN; Mary O'Driscoll, RN; Beth Tiani, 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
SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	**Ganetespib** is an HSP90 inhibitor. **Fulvestrant** is a pure anti-estrogen which is thought to act by downregulating ER expression. ***Study Design*** – Section 1.1; ***Study Rationale*** – Section 2.4; *A cycle is 28 days – Section 5.0*
Dose Calc.	• **Ganetespid** is in mg/m^2, calculated by the weight obtained at each treatment visit per DFCI standard of practice. • **Fulvestrant** is flat dosing.
Study Drug &Administration	**Ganetespid** *administration* guidelines are in Sections 5.2 and 7.1 • IV, diluted in D5W, administered over 1 hour using a 0.2µm inline filter – Section 7.1.7 • Pre-med with dexamethasone and diphenhydramine is required for Cycle 1 and 2. Participants who have successfully tolerated at least 2 cycles of ganetespid ((on Arms B or C) without a hypersensitivity reaction are not required to take the pre meds and is left to the discretion of the treating provider described in Section 5 – see alert page. Loperamide is recommended – Section 5.2.1 • Use of vascular access devices (VADs) w/***silicone catheters ONLY*** or peripheral IV is permitted. Use of VADs with catheters made of any other material is prohibited – Sect. 5.2 • Administered on Days 1, 8, and 15 of each cycle (3 weeks on, 1 week off) – Section 5.2 • See Section 5.5 for management of hypersensitivity reactions **Fulvestrant** *administration* guidelines are in Sections 5.2 and 7.2 • Administered via intramuscular injection into the buttocks on C1, D1; C1, D15; and Day 1 of each subsequent cycle; Sect. 5.2.2 & 7.2.4. NOTE: In **Arm C**, will only be administered on Day 1 of each cycle; see Alert Page
Dose Modifications and Toxicity	*Dose Modification* for *toxicity* guidelines are in Section 6 • Definition of DLT is outlined in Section 5.3 **Ganetespib**: See Section 6.2.1 for Dose Reduction Table and Dose Modification Guidelines Table • **Diarrhea is expected in most patients**. Please see Section 5.5 for diarrhea management guidelines (NOTE: These guidelines were updated on 10/2/2013). • Toxicities must recover to ≤ Grade 1 or baseline to ***resume*** treatment after skipped doses • ***Criteria to treat:*** ≤ Grade 2: ANC≥1000/mm^3; Platelets ≥50,000/ mm^3; Hgb≥8.0g/dL • Participants may continue on fulvestrant alone if treatment with ganetespib must be discontinued **Fulvestrant:** There are no dose modifications for fulvestrant - See Section 6.2.2
Concomitant Meds	*Concomitant therapy* guidelines are in Sections 5.4 and 5.5 • Participants should receive full supportive care – Section 5.5 • For participants receiving ganetespib, medications that can cause QT prolongation should be avoided – Sect. 5.4 • CYP3A4 substrates and CYP2C19 substrates should be used with caution – See Sect. 5.4 for common examples • Biphosphonate treatment is allowed if the 1st dose was given PRIOR to initiation of protocol therapy – Sect. 5.5
Required Data	*Study assessments* are in Section 9 (includes Study Calendars A-D); Appendix C • Blood for Germline DNA will be collected in a lavender top tube on C1, D1 • A CPT tube will be collected for plasma & lymphocytes – See study calendars for cohort-specific time points • Pharmacodynamic assays: PMBC collection will occur in Arms A&B on C1D1, C2D8, C2D9 & progression • Circulating tumor cells will be collected - See study calendars for cohort-specific time points • **EKG**s are single & minimal - See study calendars for cohort-specific time points; **Vital Signs** are routine.

Charting Tips	• Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record • If there is a discrepancy in the infusion time, delay in administration, or the infusion takes longer than permitted by the guidelines of the protocol, please document the reason for the discrepancy in the medical record. • Please be sure to also DOCUMENT any required observation periods, any additional vital signs, routes of administration, or injection sites

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DATE: 15 Apr 2013

TO: Robert Bradley, Sarah Mulcahey, Amy Gauger, Christine Redmond, Susan Monahan, Thomas Wilson, Shelley Mendenhall, David Noskowitz, DPD File

FROM: Neera Jain

MEMO #: DPD-9090-13-0093, Version 1

SUBJECT: Preparation and administration instructions for infusion solutions of ganetespib 300mg/vial (25mg/mL), to be included in the Pharmacy Manual.

Written by:

Neera Jain 15 Apr 2013

Neera Jain, Ph.D.
Director, Formulation R&D

Approved by:

15 Apr 2013

Thomas Hanlon
Senior Director, Drug Product Manufacturing and Supply Chain

Suresh R. Babu 15 Apr 2013

Suresh Babu, Ph.D.
Vice President, Drug Product Development

Objective

The purpose of this memo is to outline the preparation and administration instructions for ganetespib 300mg/vial (25mg/mL), to be included in the Pharmacy Manual.

Ganetespib 300mg/vial (25mg/mL)

Each vial contains 12mL of deliverable ganetespib (12.84mL total including an overage per USP requirements) at a concentration of 25 mg/mL in a polyethylene glycol 300, polysorbate 80 and dehydrated alcohol cosolvent-surfactant system. The drug product is a clear, colorless-to-pale yellow solution, essentially free of visible particles. The drug product vial can be identified with a dark blue cap and applicable label.

Ganetespib 300mg/vial (25mg/mL) Shipping:

The Ganetespib 300mg/vial drug product is shipped to sites at ambient conditions.

Ganetespib 300mg/vial (25mg/mL) Storage:

The Ganetespib 300mg/vial drug is stored at 20°C - 25°C (68°F – 77°F). If the stated storage is not available the drug product can be stored at refrigerated conditions (2°C - 8°C or 35.6°F - 46.4°F). DO NOT FREEZE.

Documenting and Reporting of Temperature Excursions during storage:

All excursions from the stated storage condition should be documented. Excursions ≤30°C (86°F) that do not exceed 48 hours or ≤40°C (104°F) that do not exceed 1 hour do not need to be reported to the sponsor. Use of the affected drug product is acceptable. All other excursions must be reported to the study sponsor for evaluation.

Preparation Instructions for Infusion Solutions using ganetespib 300mg/vial (25mg/mL)

Study drug preparation should be performed under standard aseptic conditions following chemotherapeutic precautions.

In general, the following concentration range guidelines should be used when preparing ganetespib for infusion. Sponsor-approved deviations from these recommendations may be necessary based on an individual patient's BSA and dose level. However, at no time should a concentration be outside the range of 0.1 - 1.1mg/mL. The target infusion volume should always be 500mL.

1. Calculate the total dose of ganetespib the patient is to receive based on their BSA:

   Total ganetespib dose (mg) = ganetespib dose (mg/m^2) X BSA (m^2)

2. Calculate the total volume of ganetespib drug product Solution (25mg/mL) needed:

   Total ganetespib Solution (mL) = Total ganetespib dose (mg) from Step 1 / 25mg/mL

3. Calculate the total volume of 5% Dextrose for Injection (D5W) needed for preparation of the 500mL infusion solution:

   Total D5W Volume (mL) = 500mL – Total ganetespib Solution (mL) from Step 2

4. NOTE: This step is required only for the Ganetespib 300mg/vial (25mg/mL) drug product if stored at 2-8°C.

   Remove the appropriate number of ganetespib vials from the refrigerator and let them sit at room temperature for approximately 20 minutes.

5. Into an empty 500mL non-PVC, non-DEHP containing infusion bag or a 500mL glass bottle, transfer the calculated amount of D5W in Step 3 using a syringe and a needle. The D5W volume can also be adjusted to the calculated D5W volume in Step 3 if pre-filled bags/bottles are used.

   Caution: Non-PVC, non-DEHP bags <u>must</u> be used.

6. Using a syringe and a 16G or 18G needle carefully withdraw the calculated amount of the ganetespib drug product solution from Step 2 and inject into the infusion bag containing D5W.

   Note: Because of the viscosity of the drug product a 16G needle is recommended for ease of withdrawal.

7. Carefully squeeze the transfer ports, 4 corners of the bag, and the middle of the bag to ensure complete mixing of the drug product and D5W. Continue mixing by gently inverting the bag end-over-end 10 times. Visually inspect the final infusion solution. The final infusion solution should be clear and essentially free of any visible particles.

   Caution: Do not use if a clear solution is not obtained. Contact the study Sponsor.

8. Attach a non-PVC, non-DEHP containing infusions set with a 0.22µm end-filter to the infusion bag.

Caution: Non-PVC, non-DEHP tubing with a 0.22µm filter must be used.

9. Store the infusion solution at room temperature until use, avoiding direct exposure to light. Upon completing the infusion solution preparation, the 1-hour administration of the infusion solution must be completed within 4 hours. The time needed to prepare the dosing solution does not count against the 4-hour limit. There is no need to protect the IV bag from ambient light during the infusion.

   Caution: Do not refrigerate the infusion solution.

   Caution: The 1-hour administration must be completed within 4 hours of finishing the infusion solution preparation.

10. Set up the infusion pump to deliver the 500mL ganetespib infusion solution over 60 minutes.

    Note: Use of Vascular Access Devices:

    Based on preclinical data, use of vascular access devices (VADs) (such as ports and peripherally-inserted central catheters [PICCS]) containing <u>silicone</u> catheters are permitted.

    Use of VADs with catheters made of any material other than silicone is <u>not</u> allowed.

    Following ganetespib administration through a VAD, care should be taken to flush the line after each dose of study drug. Please follow routine clinical practice for care of patients utilizing VADs.

11. At the end of the infusion, the IV tubing **must be flushed with D5W** to ensure complete delivery of the required dose of ganetespib. The infusion rate of the D5W flush should be at the same rate as the drug infusion.
12. Dispose of used IV bags, tubing and used drug vials per institutional guidelines.

Preparation and administration instructions for infusion solutions of ganetespib 300mg/vial (25mg/mL), to be included in the Pharmacy Manual.

REVISION HISTORY

Memo Number	Version	Reason for Change	Date
DPD-9090-13-0093	0	New	28Mar2013
DPD-9090-13-0093	1	Updated the storage and temperature excursion descriptions.	15Apr2013

Ganetespib Study Medication Packaging, Shipment, Receipt, Storage and Destruction

Description of Ganetespib Packaging

300 mg vials: 25 mg/mL ganetespib (STA-9090) is identified with a **dark blue cap** and the label on the vial reads **"300 mg/vial/12mL (25 mg/mL)"**. Each vial contains a total of 12 mL of deliverable STA-9090 (12.84 mL total including an overage per USP requirements) at a concentration of 25 mg/mL in a polyethylene glycol 300, polysorbate 80, and dehydrated alcohol cosolvent-surfactant system.

Medications Shipment

Synta Pharmaceuticals Corp. (Synta) will coordinate the initial shipment of ganetespib for this study. The initial shipment will occur after the necessary regulatory documents have been reviewed by Synta and a clinical trial contract is in place. When these criteria have been met, the first shipment of study medication will be approved and shipped to the designated recipient at the clinical research site.

Study Medications Receipt Procedures

1) Upon receipt of the study medications shipment, open the shipping container and visually inspect the contents. Refer to the Material Safety Data Sheet for proper handling instructions.
2) The shipping container should include study medication vials and a packing list.
3) Reconcile the shipment's contents with what is recorded on the packing slip.
4) Visually examine the shipping container, noting any damage to the box. If any vials are damaged, please contact Steve Dunlap (1-781-541-7907 or sdunlap@syntapharma.com) at Synta immediately.
5) Be sure to confirm that the shipment contains 300 mg/vials and store according to Storage Conditions outlined below.
6) When storing the study medications, use an inventory management method that ensures the oldest inventory is used first.
    - **If the study drug has expired, do not use the study medication. Contact Synta immediately and quarantine expired drug until destruction authorization is granted by Synta.**
7) Record the shipment in the Study Medications Inventory Log once inspection of the shipment has been completed. Damage or discrepancies should be reported to Synta.
8) Complete the "Acknowledgement of Receipt" portion of the Packing List and fax a completed copy to Synta at 1-866-490-9019 or email a scanned copy to IST_Drug_Order@syntapharma.com. File a copy of the Packing List in the Pharmacy Manual.

Storage Conditions

The Ganetespib 300mg/vial drug product should be stored at 20°C - 25°C (68°F – 77°F) with excursions allowed between 15°C and 30°C (59°F and 86°F) (USP Controlled Room Temperature). Alternatively, ganetespib drug product may be stored in a cool place between 8°C (46°F) and 20°C (68°F). DO NOT FREEZE. If the stated storage is not available, please contact Synta CTM (Clinical Trial Manager) immediately for appropriate instructions.

All excursions from the above stated storage condition should be documented and addressed per internal procedures. The Synta CTM must be notified of the following excursions for evaluation and disposition instructions:

- **Less than or equal to 0°C (32°F) for any duration**
- **Above 0°C (32°F) but below 8°C (46°F) for more than 48 hours**
- **Above 25°C (77°F) but below or at 40°C (104°F) for more than 48 hours**
- **Above 40°C (104°F) for any duration.**

Drug Product not affected by the excursions listed above is acceptable for use.

Study medications should be stored in a limited access, secure storage area. Daily temperature recordings should be maintained and available for review upon request. Manual recordings and electronic or mechanical records are acceptable.

Study Medications Re-supply

Study medications re-supply will be done by completing the Investigational Drug Order Form (located in the Pharmacy Manual) and emailing the signed document to IST_Drug_Order@syntapharma.com or faxing to 1-866-490-9019. Please allow 7 business days for a study medication shipment. Study medications will be shipped weekdays with the exception of Friday.

Once a re-supply shipment has been received, the Study Medication Receipt Procedures (Steps 1-8 above) must be followed.

Study Medication Disposal/Destruction Procedure

Study medication will be disposed of in accordance to institutional guidelines. All unused vials will be retained at the site until Synta personnel are notified and instructions are given. The site will provide the Synta CTM or the study monitor with a copy of the study medications disposal/destruction policy prior to any study medication being destroyed or disposed and will send a scanned copy of the Study Medications Inventory Log and destruction records to Synta upon request.