NCI Protocol #: Not Applicable

DF/HCC Protocol #: 15-530

DF/HCC Biomedical Protocol Template: June 30, 2014

TITLE: An open-label, phase Ib/II clinical trial of CDK 4/6 inhibitor, ribociclib (LEE011), in combination with trastuzumab or T-DM1 for advanced/metastatic HER2-positive breast cancer.

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NCI-Supplied Agent(s): Not Applicable

Other Agent(s):

Ribociclib, NSC#, Novartis.
Trastuzumab, NSC#, Commercial supply
Trastuzumab emtansine, NSC#, Commercial supply
Fulvestrant, NSC#, Commercial supply

IND #: 128784
IND Sponsor: Sara Tolaney, MD, MPH

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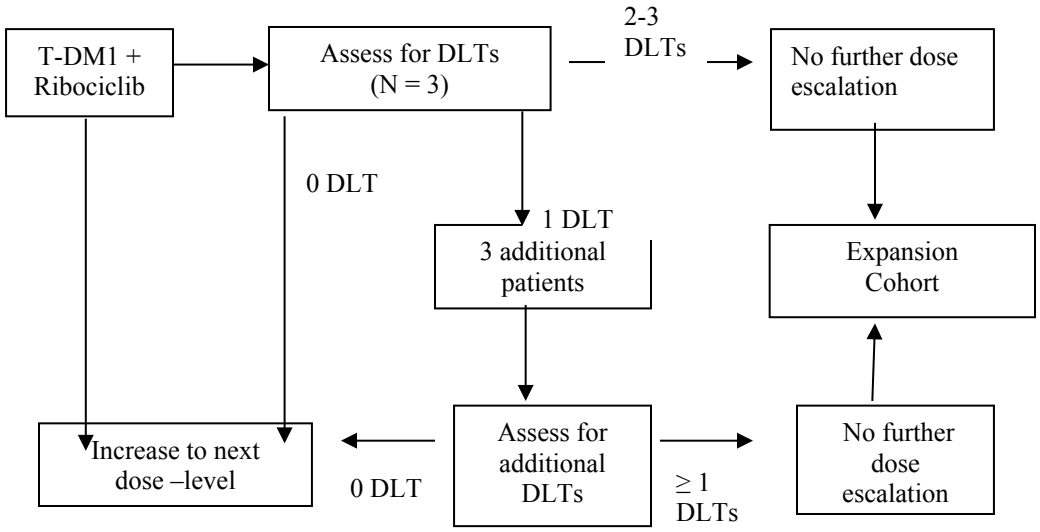
SCHEMA

COHORT A: Trastuzumab-DM1 + Ribociclib (3+3 Design)

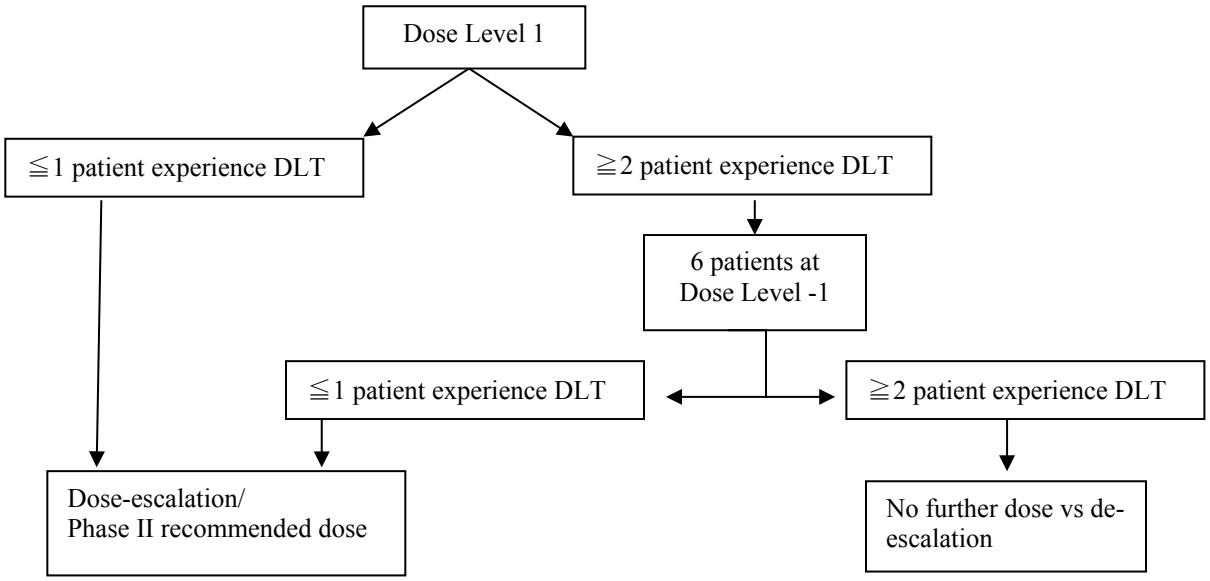


DLT: Dose-limiting toxicity; MTD: Maximum tolerated dose; R2PD = Recommended phase-2 dose.

Maximum dose-escalation of Ribociclib (LEE011) will be up to 600 mg (section 5.2). At least six evaluable patients will be treated at a dose level for an MTD/RP2D to be declared. Once an MTD/RP2D is declared, up to nine additional evaluable patients will be enrolled in the expansion cohort for a total of up to 15 evaluable patients to be treated at the MTD/RP2D.

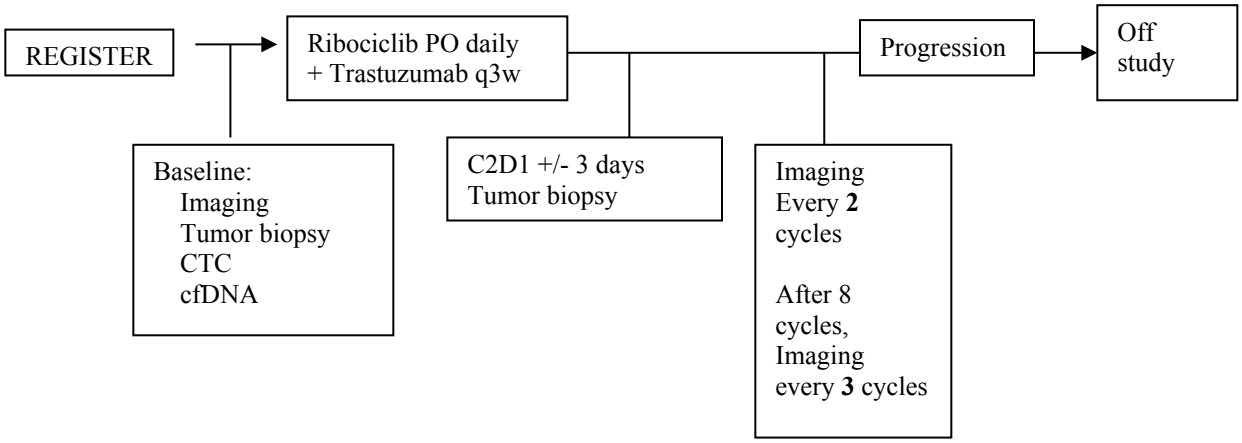
COHORT B: Trastuzumab + Ribociclib (Phase Ib and Phase II)

Phase Ib:



DLT: Dose-limiting toxicity; MTD: Maximum tolerated dose; R2PD = Recommended phase-2 dose
Maximum dose-escalation of Ribociclib (LEE011) will be up to 400 mg (section 5.2).

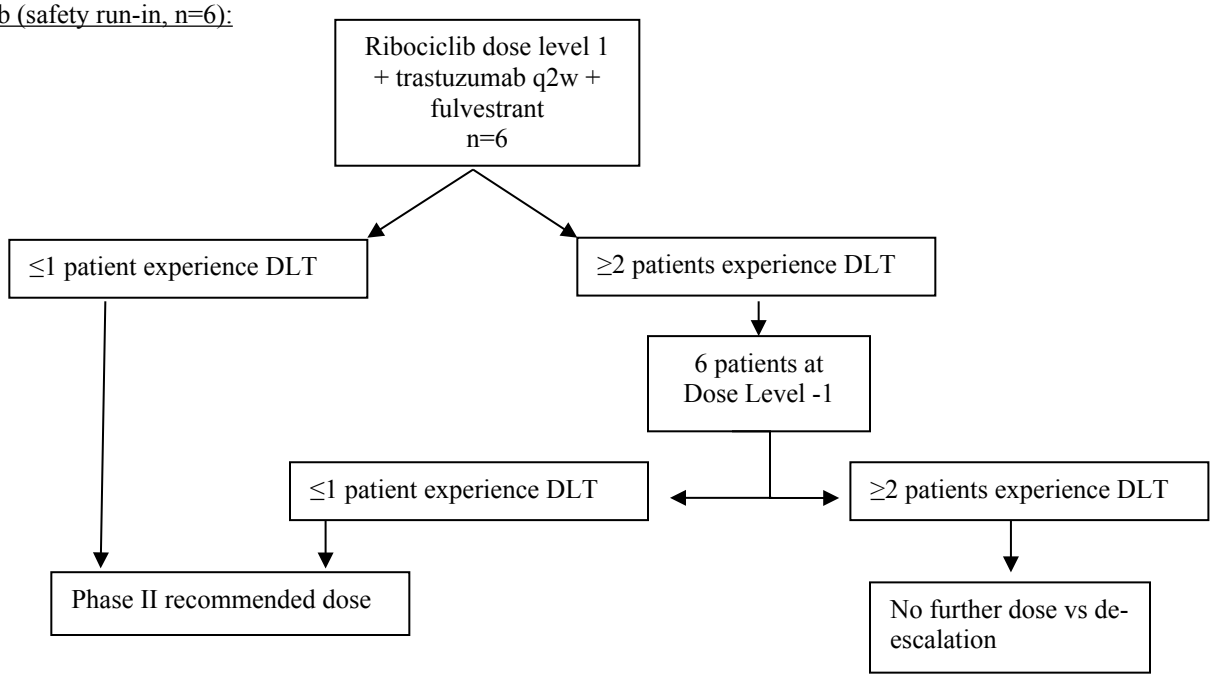
Phase II:



NB – COHORT B CLOSED TO ACCRUAL IN MARCH 2017, BECAUSE OF FAILING TO MEET STAGE I CRITERIA FOR PROGRESSING FROM STAGE I TO STAGE 2 OF THE TWO-STAGE PHASE II COMPONENT WAS DEEMED TO BE HIGH.

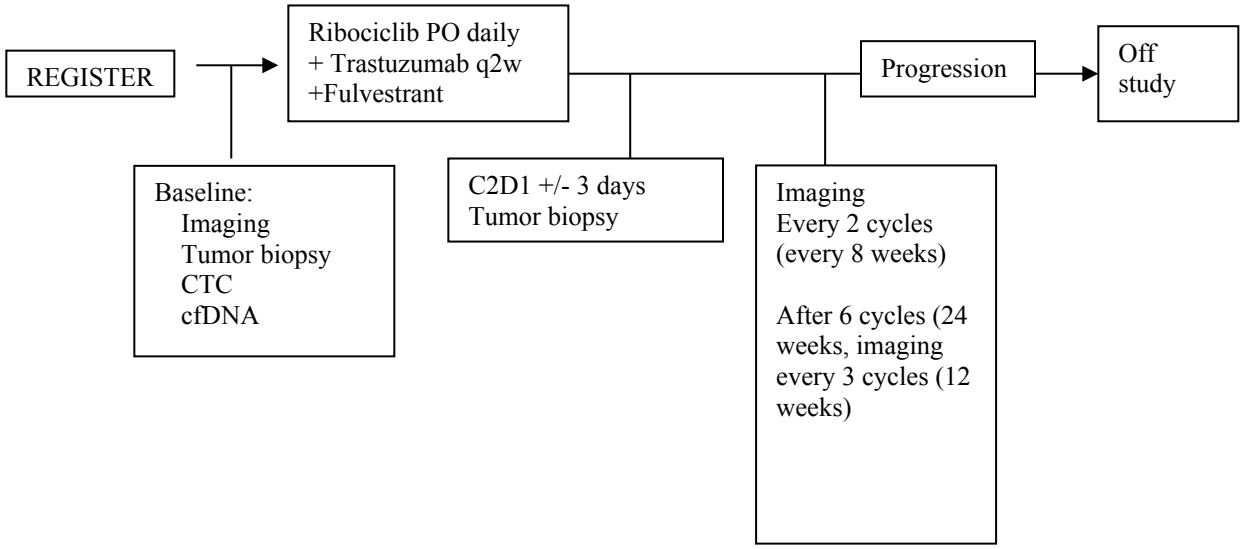
COHORT C: Trastuzumab + Ribociclib + Fulvestrant (ER+, HER2+ breast cancer) (Phase Ib and Phase II)

Phase Ib (safety run-in, n=6):



DLT: Dose-limiting toxicity; MTD: Maximum tolerated dose; R2PD = Recommended phase-2 dose
Maximum dose of Ribociclib (LEE011) will be to 400 mg continuous (section 5.2).

Phase II:



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1. OBJECTIVES

This is a phase Ib/II clinical trial designed to evaluate the safety and activity of ribociclib in combination with anti-HER2 therapies (and in Cohort C, endocrine therapy) among women with metastatic or locally advanced treatment-refractory Human Epidermal growth factor Receptor-2 (HER2)-positive breast cancer. The specific anti-HER2 therapy will be trastuzumab emtansine (T-DM1, cohort-A), or trastuzumab (cohort B).

1.1 Study Design

Cohort A will assess the safety, tolerability, and maximum tolerated dose (MTD) or recommended phase II dose (RP2D) of ribociclib in combination with T-DM l. The study will use a 3+3 dose escalation design to determine the MTD/RP2D. Three to six evaluable patients will be enrolled in each cohort in the dose escalation phase. Safety will be monitored throughout the study by the safety monitoring committee (SMC).

Cohort-B will assess the safety, tolerability, and response rate of ribociclib in combination with trastuzumab. The phase Ib component will assess safety of ribociclib in combination with trastuzumab. Accrual will be paused after the first 6 patients have been enrolled onto the study. Observation of these patients will continue until all 6 have completed 1 cycle of therapy (3 weeks) and assessed for DLTs (dose-limiting toxicities) during that cycle. Further dosing decisions would be made based as it outlined in the trial schema. Once the recommended phase II dose is confirmed, the single-arm, two stage phase II portion will begin enrolling.

ADDENDUM: COHORT B CLOSED TO ACCRUAL IN MARCH 2017, BECAUSE RISK OF FAILING TO MEET CRITERIA FOR PROGRESSION FROM STAGE 1 TO STAGE 2 OF THE TWO-STAGE PHASE II COMPONENT WAS DEEMED TO BE HIGH.

Cohort C will assess the safety, tolerability, and response rate of ribociclib in combination with trastuzumab and fulvestrant in patients with advanced HER2-positive breast cancer that is also hormone receptor positive (ER-positive and/or PR-positive). The phase Ib component will assess safety of ribociclib in combination with trastuzumab and fulvestrant. Accrual will be paused after the first 6 patients have been enrolled onto the study. Observation of these patients will continue until all 6 have completed 1 cycle of therapy (4 weeks) and assessed for DLTs (dose-limiting toxicities) during that cycle. Further dosing decisions would be made as outlined in the trial schema. Once the recommended phase II dose is confirmed, the single-arm, two stage phase II portion will begin enrolling.

1.2 Primary Objectives

The primary objectives of this study are:

- Cohort A:
    - To estimate the maximum tolerated dose (MTD) and/or recommended phase II dose (RP2D) of ribociclib in combination with T-DM1 among patients with metastatic HER2-positive breast cancer.

- Cohort B:
    - To evaluate the clinical benefit rate (CBR - CR, PR, and SD at 24 weeks) using the combination of ribociclib with trastuzumab in patients with metastatic HER2-positive breast cancer.

- Cohort C:
    - To evaluate the clinical benefit rate (CBR - CR, PR, and SD at 24 weeks) using the combination of ribociclib, trastuzumab, and fulvestrant in patients with metastatic ER-positive, HER2-positive breast cancer.

1.3 Secondary Objectives

The secondary objectives of this study are:

- Cohort A:
    - To assess the safety and tolerability of ribociclib in combination with T-DM1.

- To evaluate the PK profile of ribociclib in combination with T-DM1.
- To explore clinical activity (i.e objective response rate and progression-free survival) in patients with metastatic HER2-positive breast cancer treated with trastuzumab emtansine (T-DM1) and ribociclib.
- To assess potential biomarkers of response to ribociclib in combination with T-DM1.

- Cohort B:
  - To evaluate the objective response rate (as defined by RECIST 1.1) in patients with metastatic HER2-positive breast cancer treated with trastuzumab and ribociclib.
  - To evaluate progression-free survival (PFS) and overall survival (OS) in patients with metastatic HER2-positive breast cancer treated with trastuzumab and ribociclib.
  - To assess the safety and tolerability of ribociclib in combination with trastuzumab.
  - To assess potential biomarkers of response to ribociclib in combination with trastuzumab.

- Cohort C:
  - To evaluate the objective response rate (as defined by RECIST 1.1) in patients with metastatic ER-positive, HER2-positive breast cancer treated with trastuzumab, ribociclib, and fulvestrant.
  - To evaluate progression-free survival (PFS) and overall survival (OS) in patients with metastatic ER-positive, HER2-positive breast cancer treated with trastuzumab, ribociclib, and fulvestrant.
  - To assess the safety and tolerability of ribociclib in combination with trastuzumab and fulvestrant.
  - To assess potential biomarkers of response to ribociclib in combination with trastuzumab and fulvestrant.

2. BACKGROUND

2.1 Study Disease(s)

Despite the availability of multiple effective therapies for HER2-positive breast cancer, almost all patients with advanced/metastatic HER2 positive breast cancer will experience disease progression and death. Typically, such patients will receive agents including trastuzumab, lapatinib, pertuzumab, and trastuzumab emtansine (T-DM1), in addition to cytotoxics and hormonal therapies. These therapies have changed the natural history of HER2-positive disease such that there now exists a large cohort of patients that has progressed on standard treatment, retains a good performance status, and is left without treatment options. It is estimated that approximately 10,000 patients will be diagnosed each year with metastatic HER2-positive breast cancer in the USA alone[1].

2.2 Study Rationale

In the mammalian cell cycle, entry into S phase is achieved by cyclin-dependent kinases 4 and 6 (CDK4/6), which activate a family of E2F transcription factors by phosphorylating and deactivating the retinoblastoma protein (Rb). D-cyclins are the positive regulators of these kinases, while the p16 protein encoded by the INK4a gene (cyclin-dependent kinase inhibitor 2A; CDKN2A) inhibits the kinase activity (Fig 2-1)[2]. Several lines of evidence suggest that increased CDK4/6 activity contributes to tumorigenesis. First, signal transduction pathways such as the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) that are often activated in tumors, promote cell proliferation by increasing the expression of D-cyclins, which in turn activate CDK4/6. Second, a wide range of human tumors harbor genetic aberrations that increase the activity of CDK4/6. These genetic aberrations include translocation, amplification and over-expression of D-cyclins, amplification of CDK4/6, mutations in CDK4 that render the protein resistant to p16 binding, and p16 loss due to mutation and/or deletion or the INK4a gene. Agents that inhibit the activity of CDK4/6 may be able to slow or stop the proliferation of these cancers and thereby function as effective anti-cancer drugs.

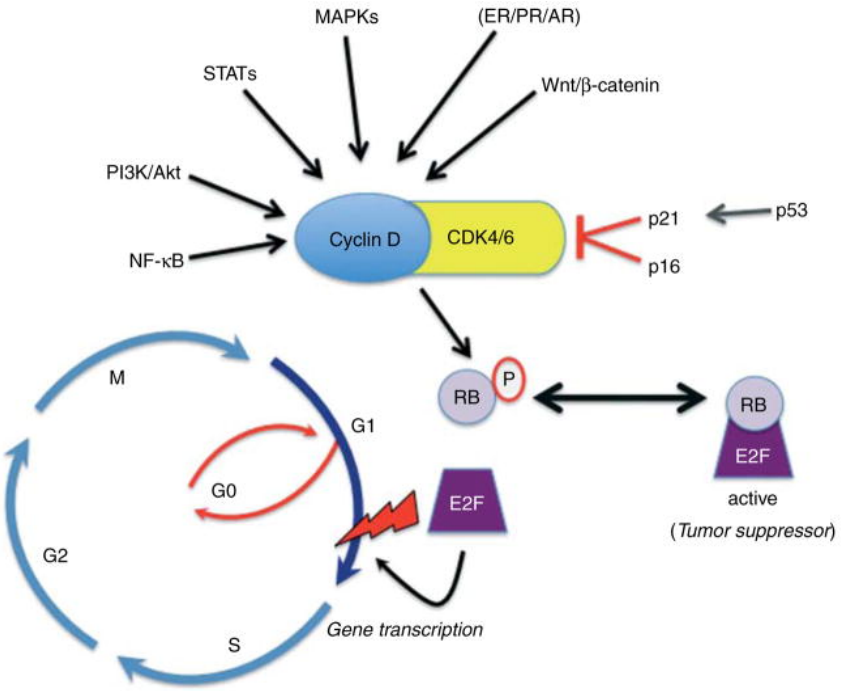

Figure 2-1 **Regulation of cell cycle checkpoint control**

Mitogenic signals converge at the level of cyclin D1 upregulation and CDK4/6 association, localization, and kinase activity. CDK4/6 phosphorylates and inactivates retinoblastoma (RB) tumor suppressor proteins, leading to dissociation of E2F transcription factors and transcriptional regulation of genes important for G1/S transition and cell cycle progression through the restriction point (lightning bolt). Ribociclib is a highly specific inhibitor of CDK4/6 which blocks the cell cycle in the G1 phase[2].

A wide range of human tumors, including breast cancer, harbor genetic aberrations that increase the activity of CDK4/6. Furthermore, focal copy number abnormalities that result in increased CDK activity are among the most commonly described mutations observed in diverse tumor types; these mutations include amplifications of the genes that encode cyclin D1 or CDK4, and deletions affecting the CDKN2A locus, which encodes the $p16^{INK4a}$ inhibitor of CDK activity[3]. Finally, the retinoblastoma protein (Rb) is a tumor suppressor protein that is dysfunctional in several major cancers[4]. Direct analyses of primary tumors have revealed loss of Rb expression in 20-35% of tumors, and loss of heterozygosity or other alterations of the Rb locus in 7.4% of tumors[5–8].

Cell cycle related genes and proteins are frequently deregulated in breast cancer. Approximately 15-20% of human breast cancers exhibit amplification of the cyclin D1 (CCNDl) gene[9–11] while the majority of human mammary carcinomas overexpress CCNDl protein[12–15]. Overexpression of CCND1 is seen early in breast cancer, and is maintained at all stages of breast cancer progression, including in metastatic lesions[12–14]. Additionally, the continued presence of CDK4-associated kinase activity is required to maintain breast tumorigenesis[16].

In preclinical studies, the cyclin D1-CDK4 axis has been shown to serve as a critical mediator of both the initiation and maintained growth of HER2-positive breast tumors[17,18], acting in a kinase-dependent fashion. Furthermore, using a novel transgenic mouse model of inducible HER2-driven mammary carcinoma, we have shown that tumor cells that resist apoptosis in the face of complete HER2-pathway blockade selectively overexpress cyclin D1. Finally, tumors that later recur in our mouse model after genetic withdrawal of HER2 show selectively high expression of cyclin D1 and CDK4 (Goel et al, unpublished).

Therefore, the existing preclinical data suggests that the cyclin D1-CDK4/6-Rb axis is an attractive therapeutic target in HER2-positive breast cancer. Using a pharmacological approach, our group and others have shown that HER2-positive breast cancer cell lines are frequently sensitive to CDK4/6 inhibition[19], and that a number of HER2-positive lines that are resistant to conventional anti-HER2 therapies retain sensitivity to CDK4/6 inhibition. Additionally, our preliminary data

suggests that heightened activity through the cyclin D1-CDK4 axis mediates resistance to anti-HER2 therapy (Goel et al, unpublished). Consistent with this, using a panel of 6 HER2-positive cell lines we have observed that the combination of anti-HER2 therapy (trastuzumab or lapatinib) plus CDK4/6 inhibition confers significantly higher cytotoxicity than either agent alone. Additional studies with combination of CDK 4/6 inhibitors, including ribociclib with other anntiHER2 therapies such as T-DM1, have shown synergistic effect (personal communication with Novartis).

Rationale for opening Cohort C:

At the time this protocol was initially activated, limited clinical data was available demonstrating the efficacy of CDK4/6 inhibitors in HER2-positive breast cancer[20]. Two cohorts were proposed: Cohort A, a phase 1 study of ribociclib together with the anti-HER2 antibody drug conjugate T-DM1 (comprising trastuzumab linked to a potent cytotoxic); and Cohort B, a phase 1b/2 study of ribociclib together with trastuzumab. In March 2017, Cohort B was closed to accrual as the chance of meeting the efficacy criteria for progressing from Stage 1 to Stage 2 of the phase 2 study were deemed to be very low. Several patients recruited to this Cohort had HER2-positive, ER-negative breast cancer and nearly all were heavily pre-treated (median number of prior lines of therapy for advanced disease = 6). A total of 6 subjects were enrolled to the Cohort B safety-run in and no DLTs were identified. As such, the Cohort B dose expansion opened to enrollment, in which 7 patients were registered. Of the 13 patients that were treated on Cohort B, only 3 subjects had a best response of stable disease and the remaining subjects had a best response of progressive disease. In reviewing toxicity data, there were no unexpected signals identified from any of these patients.

Shortly after activation, preliminary reports of other CDK4/6 inhibitors (in particular palbociclib) in conjunction with trastuzumab in patients with HER2-positive breast cancer emerged. For example, Dr DeMichele (University of Pennsylvania) presented a 25% response rate in patients with advanced HER2-positive breast cancer treated with palbociclib and trastuzumab. Interestingly, all of these responses were observed in patients with ER-positive, HER2-positive breast cancer (SABCS 2016). Similarly, unpublished data suggested that responses to the abemaciclib-trastuzumab combination were restricted to patients whose tumors were also ER-positive. Finally, a neoadjuvant study of palbociclib, trastuzumab, pertuzumab, and fulvestrant demonstrated a pathologic complete response rate of 27% in patients with ER-positive, HER2-positive breast cancer (Gianni, SABCS 2016).

The greater activity of CDK4/6 inhibitors in HER2-positive tumors that are also ER-positive has been observed consistently in early reports, as described above. This might relate to the fact that these tumors are more likely to show luminal biology, a marker of sensitivity to CDK4/6 inhibition, than their ER-negative counterparts (Prat, SABCS 2016). Given these reports, this trial has been amended to include a new cohort (Cohort C). This cohort differs from Cohort B in the following ways:

- Restricted to patients with ER-positive, HER2-positive breast cancer
- Addition of hormonal therapy (fulvestrant) to trastuzumab and ribociclib for all patients
- Maximum number of prior lines = 5 (in cohort B this was unrestricted)
- New statistical considerations to reflect the different patient population (see section on statistical considerations)

2.3 IND Agents

2.3.1 Ribociclib

Ribociclib is an orally bioavailable, highly selective small molecule inhibitor of CDK4/6 that induces G1 arrest at sub-micromolar concentrations in a variety of Rb- positive cancer cells *in vitro*. Furthermore, *in vivo* treatment with well-tolerated doses of ribociclib led to tumor regressions in rats bearing the Jeko-1 mantle cell lymphoma (MCL) model, which harbors the t(11;14) cyclin D1 (CCND1) translocation. Ribociclib has also proven efficacious when combined with other targeted therapies *in vitro* and *in vivo* in cancers driven by a variety of oncogenic signaling pathways. Ribociclib may therefore be an effective anti-cancer agent in a variety of Rb-positive human neoplasms, especially in those that contain an activated CDK4/6-Rb pathway.

2.3.1.1 **In vitro pharmacology**

Ribociclib inhibits the CDK4/CCND1 and CDK6/CCND3 enzyme complexes with concentration resulting in 50% inhibition (IC50) values of 0.01 and 0.039 μM in biochemical assays, respectively. In Jeko-1 cells, the compound inhibits CDK4/6-dependent Rb phosphorylation with an average IC50 of 0.06 μM. Consistent with the observed inhibition of Rb phosphorylation, ribociclib also inhibited G1 to S phase cell cycle progression in Jeko-1 cells as judged by both the inhibition of bromodeoxyuridine (BrdU) uptake (IC50 of 0.1 μM) and fluorescence activated cell sorting (FACS) analysis (half-maximal increase in cells in G1 at 0.11 μM).

The effect of ribociclib on Rb phosphorylation, BrdU uptake and cell cycle progression has been assessed in > 40 cell lines derived from hematological, esophageal, liposarcoma and breast cancers. In Rb+ cell lines, ribociclib inhibits Rb phosphorylation with a median IC50 value of 0.275 μM (range: 0.06 to 8.8 μM). Similarly, ribociclib interferes with G1 to S phase cell cycle progression in these cells as determined by either BrdU uptake or FACS analysis with a median IC50 value of 0.46 μM. In contrast, in lineage-matched Rb-negative cell lines no effect of ribociclib on either Rb phosphorylation or cell cycle progression is observed. Thus, ribociclib is able to impact cell cycle progression in cell lines derived from a variety of tumor types that harbor a diversity of genetic alterations in a manner dependent on intact Rb.

2.3.1.2 **In vivo pharmacology**

Ribociclib was well-tolerated in mice and rats with body weight loss not exceeding 12.5% at doses up to 250 mg/kg qd po or 150 mg/kg qd po, respectively, for up to 28 days. However, myelosuppression was observed and correlated with Rb phosphorylation inhibition.

Treatment with ribociclib resulted in tumor regression in the Jeko-1 MCL xenograft model at doses greater than or equal to 75 mg/kg, qd po. *In vivo* pharmacokinetics (PK)/pharmacodynamics (PD) studies demonstrated dose-related inhibition of Rb phosphorylation in tumors, with continuous dosing over at least 3-5 days being required to achieve optimal target inhibition. In male nude rats, a PK/PD/efficacy study indicated that plasma levels corresponding to approximately 0.5-4μM over a 24 h dose interval are sufficient to obtain near complete inhibition of Rb phosphorylation and complete regression in the Jeko-1 MCL xenograft model.

Ribociclib has demonstrated *in vivo* anti-tumor activity in subsets of tumor xenograft models. Consistent with the compounds mechanism of action, efficacy was only observed in tumors expressing Rb. Tumor types where ribociclib has demonstrated robust anti-tumor activity include but are not limited to breast, melanoma, neuroblastoma, malignant rhabdoid, lung, pancreas and hematological malignancies.

In addition, ribociclib has shown anti-tumor activity when combined with targeted agents which inhibit signaling pathways known to regulate D-cyclin levels, including inhibitors of the RAF/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PIK3) and mammalian target of rapamycin (mTOR) pathways.

2.3.1.3 **Safety pharmacology and toxicology**

In vivo cardiac safety studies demonstrated a signal for QT prolongation with the potential to induce incidences of premature ventricular contractions (PVCs) at higher exposure levels.

The effects of ribociclib on the bone marrow (hypocellularity), lymphoid system (lymphoid depletion), intestinal mucosa (atrophy), skin (atrophy), bone (decreased bone formation) and testes (atrophy) are considered to be related to the pharmacological inhibition of cell replication in these tissues due to CDK4/6 inhibition. An increased number of ovarian corpora lutea was observed in a single female dog in the 4-week toxicity study at the highest dose tested (20 mg/kg/day) and this effect could also be related to the pharmacology of ribociclib (arrest of estrous cycle). The liver, bile system and gall bladder (proliferative changes, cholestasis, sand-like gallbladder calculi and inspissated bile) and the kidney (concurrent degeneration and regeneration of tubular epithelial cells) were identified as additional target organs of toxicity which are not likely related to the primary pharmacology of ribociclib. Inflammatory changes in the lungs of dogs were

considered secondary to aspiration of test- article and are indicative of the irritant potential of the formulated test-article in the respiratory tract. Correlating hematological and/or biochemistry changes were seen for the effects described in the bone marrow, lymphoid system and liver. Generally all changes demonstrated either reversibility or a clear tendency towards reversibility.

2.3.1.4 **Nonclinical pharmacokinetics and metabolism of ribociclib**

Ribociclib showed high clearance (CL) in the mouse, rat, dog and monkey. The volume of distribution was large across species and the terminal elimination half-life (T1/2) was moderate in rodents and monkey (~2 to 5 h) and longer in dog (18 h).

Bioavailability was low to moderate in rat (37%) and cynomolgus monkey (17%), and moderate in mouse (65%) and dog (64%). Following oral administration, time to reach maximal plasma concentrations (Tmax) occurred between 2 to 4 h across species. Gender- dependent toxicokinetics were observed in rats with higher exposure to ribociclib in males as compared to females and with higher exposure to the metabolite, LEQ803.

Plasma protein binding was moderate in all species (unbound fraction (fu) in human: 30%).

In a rat ADME (absorption, distribution, metabolism and excretion) study, extensive distribution of [^{3}H]ribociclib and its metabolites was seen. In pigmented rats, radioactivity was specifically found in melanin-containing structures, and the highest exposure to total radiolabeled components was observed in eye ciliary body, eye choroid, meninges, tactile hair and hair follicles. Radioactivity was not detected in the brain. Tlast (last observation timepoint) was ≤ 48h for most tissues, but long (168 to 840h) for lymph nodes, preputial gland, testis, eye and meninges. At one week ≤ 0.04% of the dose was retained in the carcass.

LEQ803 (N-demethylation) was a prominent metabolite found in mouse, rat, dog, monkey and human hepatocytes. This metabolite retains some pharmacologic activity and interacts with human Ether-a-go-go Related Gene (hERG) channels in vitro. In male rats, unchanged ribociclib (24.7% of [^{3}H]AUC0-24h) and its metabolite M11 (26.3% of [3H]AUC0-24h) were the major components in plasma. In rats, ribociclib was eliminated mainly by metabolism. The major metabolism pathway was direct sulfation of ribociclib to M8 and its excretion into the bile. Direct ribociclib secretion accounted for 18.2% of the total plasma clearance.

Results from the ADME (male rats) study showed that 3H-components were predominantly excreted with bile (61.4% of dose). Minor urinary excretion was observed (5.9% of dose after p.o.). The majority of the administered dose (87.3%) was excreted within 24 h via urine, feces (enteric secretion) and bile.

In vitro, ribociclib was a reversible inhibitor of cytochrome P450 (CYP) enzymes CYP1A2, CYP2E1 and CYP3A4 and a time-dependent inhibitor of CYP3A4. ribociclib may inhibit these enzymes under therapeutic conditions. No pregnane X-receptor (PXR)-mediated CYP3A4 induction was observed. The *in vitro* inhibitory potency of ribociclib observed for the transporters OATP1B1 (organic anion transporting polypeptide 1B1), BCRP (breast cancer resistance protein), OCT1 (organic cation transporter 1), OCT2, MATE1 (multidrug and toxin extrusion protein 1), MATE2K and BSEP (bile salt export pump) may translate into clinically relevant inhibition at therapeutic doses.

Elimination of ribociclib is dominated by oxidative metabolism mainly via CYP3A4 with a minor contribution by flavin-containing monooxygenase 3 (FMO3). The elimination of ribociclib may be affected by co-administered drugs that inhibit or induce CYP3A4. Although ribociclib is a substrate of the P-glycoprotein (P-gp) efflux transporter and subject to active uptake into hepatocytes, these processes are likely not clinically relevant due to the high passive permeability of ribociclib.

2.3.1.5 **Clinical experience with ribociclib**

Ribociclib is currently being investigated in patients as a single agent in 3 phase I studies: CLEE011X1101, CLEE011X2101, CLEE011X2102 and in combination in 10 studies: 8 phase Ib/II CLEE011X2105, CLEE011X2106, CLEE011X2107, CLEE011X2108, CLEE011A2112C, CMEK162X2114, CMEK162X2110, CLGX818X2102, a randomized phase II CLEE011A2201, and a randomized phase III CLEE011A2301. Ribociclib is also being investigated in 3 clinical pharmacology studies in healthy subjects: CLEE011A2111, CLEE011A2101, and CLEE011A2106.

Single agent experience:
In single agent trials, a total of 179 patients have been treated: 132 in study CLEE011X2101 (in a Caucasian population, including 85 in the dose escalation), 15 in CLEE011X1101 (in Japanese patients, all in the dose escalation) and 32 in CLEE011X2102 (in patients under the age of 21 years, all in the dose escalation).

A total of 18 patients presented toxicities meeting the dose limiting toxicity (DLT) criteria (10 in CLEE011X2101, 4 in CLEE011X1101 and 4 in CLEE011X2102): these consisted of Grade 3 stomatitis, Grade 3 pulmonary embolism, Grade 3 hyponatremia, prolonged Grade 3/4 neutropenia (x2), prolonged Grade 2 elevated creatinine, Grade 4 thrombocytopenia (x5), Grade 3 asymptomatic QTcF prolongation with Grade 3 neutropenia, Grade 4 febrile neutropenia, Grade 3 febrile neutropenia (x2), Grade 3 electrocardiogram QT prolonged, Grade 3 fatigue, and Grade 3 asymptomatic QTcF prolongation with grade 4 neutropenia.

The maximum tolerated dose (MTD) and recommended dose for expansion (RDE) from study CLEE011X2101 were declared as 900 mg qd and 600 mg qd on a 3 weeks on/1 week off schedule, respectively. At the RDE, the most common (in at least 2 patients) adverse events (AEs) related to study treatment were (all grades, Grade 3/4): neutropenia (46.3%, 28.4%), leukopenia (46.3%, 19.4%), nausea (44.8%, 1.5%), thrombocytopenia (34.3%, 9%), fatigue (32.8%, 3%), anemia (28.4%, 3%), diarrhea (26.9%, 3%), lymphopenia (22.4%, 17.9%), electrocardiogram QT prolonged (9%, 0%), hyponatremia (3%, 1.5%), and febrile neutropenia (1.5%, 1.5%). Preliminary data for clinical activity from study CLEE011X2101 show that out of 114 evaluable patients, 3 partial responses were seen at the 600 mg qd dose level: one in a BRAF/NRAS wild type, CCDN1 amplified melanoma patient, one in a CDKN2A loss head and neck acinar carcinoma patient, and one in an ER+/HER2-, PIK3CA mutant, CCDN1 amplified breast cancer.

Table 2-1 **Serious adverse events with a suspected causal relationship with LEE011 single agent**

Serious suspected adverse events which have occurred with LEE011 (single agent)	**System Organ Class Preferred Term Preferred Term**
Blood and lymphatic system disorders	Anaemia, Febrile neutropenia, Neutropenia, Thrombocytopenia
Gastrointestinal disorders	Diarrhea, Nausea
General disorders and administration site conditions	Generalized edema
Infections and infestations	Herpes simplex
Investigations	Blood creatinine increased

As of 28-Mar-2014, PK data were available from approximately 128 patients from the first-in- human (FIH) study CLEE011X2101. Following oral dosing, ribociclib was rapidly absorbed with median Tmax ranging from 1 to 5 hours. ribociclib plasma exposure exhibited slightly over-proportional increases in exposure across the dose range tested (50 to 1200 mg), with no clear evidence of time-dependent auto-inhibition of its clearance mediated by CYP3A4. Steady-state was generally reached by Day 8 and the mean effective T1/2 based on accumulation ratio (i.e., T1/2,acc) ranged from 15.9 to 32.6 hours across the dose range tested. The accumulation ratio based on AUC obtained in a dosing interval (Racc) across the studied doses ranged from 1.55 to 2.52.

The MTD and RDE are still under evaluation for study CLEE011X1101 at the dose of 600 mg qd. In study CLEE011X2102, the MTD was determined to be 470 mg/m2 qd and the RDE was determined to be 350 mg/m2 qd on a 3 week on/1 week off schedule in pediatric patients.

A food effect study conducted in healthy subjects CLEE011A2111 indicated that ribociclib administered as drug-in-capsule (DiC) can be taken without regard to meals.

A drug-drug interaction (DDI) study with ritonavir (a strong CYP3A4 inhibitor) and rifampicin (a strong CYP3A4 inducer) conducted in healthy subjects CLEE011A2101 indicated that concurrent use of strong CYP3A4 inhibitors or strong CYP3A4 inducers may markedly affect ribociclib exposure and should be avoided.

A DDI cocktail study with midazolam (a sensitive CYP3A4 substrate) and caffeine (a sensitive CYP1A2 substrate) was conducted in healthy subjects CLEE011A2106. Preliminary PK data indicate that ribociclib (400 mg) is a moderate inhibitor of CYP3A4, but did not have a substantial effect on CYP1A2 substrates in humans. Concurrent use of sensitive CYP3A4 substrates with a narrow therapeutic index should be avoided. Concurrent use of CYP1A2 substrates is not expected to lead to clinically important DDIs.

CLEE011X2101 study is currently evaluating a dosing schedule with ribociclib 400mg continuous dosing.

Combination trial experience:
Ribociclib is being evaluated in several combination trials: letrozole (CLEE011A2201, CLEE011A2301]), letrozole and BYL719 CLEE011X2107, letrozole and buparlisib CLEE011A2112C, fulvestrant and buparlisib CLEE011X2108, everolimus and exemestane CLEE011X2106, LGX818 (CLEE011X2105, CLGX818X2102), MEK162 CMEK162X2114, or MEK162 and LGX818 CMEK162X2110. Of note, one randomized phase 3 trial MONALEESA-2) has now been reported and demonstrates a significant improvement in progression-free survival when ribociclib is added to first line aromatase inhibitor therapy in patients with advanced, ER-positive, HER2-negative breast cancer.

2.3.2 **Trastuzumab Emtansine (T-DM1)**

T-DM1 is a HER2-targeted antibody and microtubule inhibitor conjugate indicated for the treatment of patients with HER2+ metastatic breast cancer who previously received trastuzumab and a taxane, either separately or in combination.

2.3.2.1 **Efficacy of T-DM1**
T-DM1 is a HER2-targeted antibody and microtubule inhibitor conjugate indicated for the treatment of patients with HER2+ metastatic breast cancer who previously received trastuzumab and a taxane, either separately or in combination.

2.3.3 Efficacy of T-DM1

The efficacy of T-DM1 was evaluated in a randomized, multicenter, open-label study of 991 patients with HER2+, unresectable locally advanced or metastatic breast cancer[21]. This Phase 3 study showed an improvement in OS and PFS comparedto capecitabine and lapatinib.

In addition, there is increasing anecdotal evidence that T-DM1 may have some activity in the CNS with decrease in size of CNS metastases noted in some patients[22,23]. In the EMILIA study of T-DM1 vs. lapatinib plus capecitabine, patients with CNS metastasis at baseline treated with T-DM1 had a median OS of 26.8 months, compared to 12.9 months for similar patients treated with capecitabine plus lapatinib[24].

2.3.4 Safety of T-DM1

The most common (frequency ≥ 25%) AEs seen in patients treated with T-DM1 in clinical studies have been fatigue, nausea, musculoskeletal pain, thrombocytopenia, headache, increased transaminases, and constipation. The most common ≥ Grade 3 adverse drug reactions (frequency > 2%) were thrombocytopenia, increased transaminases, anemia, hypokalemia, peripheral neuropathy, and fatigue (KADCYLA™ package insert).

Patients treated with T-DM1 are at increased risk of developing left ventricular dysfunction. A decrease of left ventricular ejection fraction (LVEF) to < 40% has been observed in patients treated with T-DMl. In a randomized study, left ventricular dysfunction occurred in l.8% of patients in the T-DM l -treated group and 3.3% of patients in the lapatinib plus capecitabine-treated group[21].

Management of increased serum transaminases, hyperbilirubinemia, left ventricular dysfunction, thrombocytopenia,

pulmonary toxicity, or peripheral neuropathy may require temporary interruption, dose reduction, or treatment discontinuation of T-DMl as per guidelines provided in the package insert.

The efficacy of T-DM1 was evaluated in a randomized, multicenter, open-label study of 991 patients with HER2+, unresectable locally advanced or metastatic breast cancer[21]. This Phase 3 study showed an improvement in OS and PFS compared to capecitabine and lapatinib.

In addition, there is increasing anecdotal evidence that T-DM1 may have some activity in the CNS with decrease in size of CNS metastases noted in some patients[22,23]. In the EMTLIA study of T-DM1 vs. lapatinib plus capecitabine, patients with CNS metastasis at baseline treated with T-DM1 had a median OS of 26.8 months, compared to 12.9 months for similar patients treated with capecitabine plus lapatinib[24].

2.3.5 **Trastuzumab**

Trastuzumab is a humanized anti-HER2 antibody that binds to subdomain IV of the HER2 extracellular domain and exerts its antitumor effects by blocking HER2 cleavage, stimulating antibody-dependent, cell-mediated cytotoxicity and inhibiting ligand-independent, HER2-mediated mitogenic signaling[25].

2.3.5.1 **Summary of PK profile and metabolism for trastuzumab**

A Phase I single dose study (H0407g) of intravenous trastuzumab infusions ranging from 10-500 mg resulted in dose-dependent PK with serum clearance of trastuzumab decreasing with an increasing dose at doses <250 mg. PK modeling of trastuzumab concentration-time data from 7 patients that were administered doses of 250 mg and 500 mg had a mean half-life of 5.8 days (range 1-32 days). Additionally, PK modeling showed that weekly trastuzumab doses ≥250 mg resulted in serum trough levels of >20 g/mL that was above the minimum effective concentration observed in preclinical xenograft studies in tumor-bearing mice. The Phase I data supported the weekly dosing schedule that was implemented in all subsequent Phase II and Phase III clinical trials. A weight-based dose schedule was adopted after two Phase II trials (H055 l g and H0552g) suggested that inter-subject variability in trastuzumab PK was related to body weight. These findings resulted in a trastuzumab dose schedule of a 4 mg/kg loading dose followed by a weekly 2 mg/kg maintenance dose which was utilized in the two pivotal Phase III trials (H0648g and H0649g). These studies were the basis of the FDA approval of trastuzumab for HER2-positive MBC.

The trastuzumab PK data from studies H0407g (Phase I), H055 l g (Phase II), and H0649 (pivotal) have been subsequently reanalyzed by a population PK approach using nonlinear mixed effect modeling (NONMEM). A linear two-compartment model best described the concentration-time data, and accounted for the accumulation of trastuzumab serum concentrations seen in the Phase II and Phase III clinical studies. A covariate analysis was conducted using the subjects from these single agent studies to evaluate the effect of pathophysiologic covariates (e.g. age, weight, shed antigen) on the PK parameter estimates. The covariates, that significantly influenced clearance, were the level of shed antigen and the number of metastatic sites. Volume of distribution was significantly influenced by weight and shed antigen level. Additionally, data from the Phase III study, H0648g, were added to assess the influence of concomitant chemotherapy on trastuzumab PK. Importantly, chemotherapy (AC or paclitaxel) did not significantly alter trastuzumab PK. The estimated half-life of trastuzumab based on the final model was 28.5 days.

Analysis of data obtained from two Phase II studies which utilized a loading dose of 8 mg/kg trastuzumab followed by a 6 mg/kg maintenance dose administered every 3 weeks (q3 week) as a single-agent[26], and in combination with paclitaxel (175 mg/m2)[27], confirmed that a two- compartment model best describes the PK of trastuzumab. Model-independent analysis of the data obtained in these studies gives comparable PK parameter estimates to those obtained by the population PK model, thus confirming the validity of the population PK model. In addition, the population PK model adequately predicted trastuzumab serum concentrations obtained independently in these studies. After two treatment cycles, trastuzumab exposure were similar to those measured in the once weekly dosing regimen used in the pivotal trials. Trough levels were in excess of the targeted serum concentrations established from preclinical xenograft models, and as expected, peak levels were greater than those observed upon weekly administration. The apparent half-life of trastuzumab in these studies was determined to be approximately 21 days, and the PK was supportive of a q3 week dosing schedule.

The efficacy and safety results from these Phase II studies with q3 week dosing do not appear to be different from those with weekly dose-schedules[26,28,29]. In the trastuzumab q3 weekly monotherapy study[26], 105 patients with HER2-positive MBC were treated, with an ORR of 19% (23% in patients with measurable centrally confirmed HER2-positive disease). The median baseline left ventricular ejection fraction (LVEF) was 63%, which did not significantly change during the course of the study. One patient experienced symptomatic congestive heart failure (CHF), which resolved with medical treatment for CHF and discontinuation of trastuzumab. In the study of q3 weekly trastuzumab and paclitaxel[27], 32 patients were treated with an investigator-assessed response rate of 59%. Ten patients had a decrease in LVEF of 15% or greater. One patient experienced symptomatic CHF, which improved symptomatically after medical therapy for CHF and discontinuation of trastuzumab.

More recently, experience with dosing trastuzumab every 2 weeks has also been reported. For example, the Hellenic Oncology Research Group conducted an adjuvant trastuzumab study in which trastuzumab was given every 14 days (6mg/kg loading dose then 4mg/kg afterwards). No unexpected safety or efficacy signals were obtained (Mavroudis, Annals of Oncology 2015). This regimen has typically been used when the schedule aligns with schedules of other concomitant therapies.

2.3.5.2 **Summary of safety data for trastuzumab**

Experience with trastuzumab administration has shown that the drug is relatively safe. The most significant safety signal observed during clinical trials was cardiac dysfunction (principally clinically significant heart failure [CHF]), particularly when trastuzumab was given in combination with an anthracycline-containing regimen. Much of the cardiac dysfunction was reversible on discontinuation of trastuzumab.

In addition, during the first infusion with trastuzumab, a symptom complex most commonly consisting of fever and/or chills was observed in approximately 40% of patients. The symptoms were usually mild to moderate in severity and controlled with acetaminophen, diphenhydramine, or meperidine. These symptoms were uncommon with subsequent infusions. However, in the post approval setting, more severe adverse reactions to trastuzumab have been reported. These have been categorized as hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events. Rarely, these severe reactions culminated in a fatal outcome.

Trastuzumab appears to be relatively nonimmunogenic. Only 1 of 903 patients evaluated developed neutralizing antibodies to trastuzumab. The development of anti-trastuzumab antibodies in this patient was not associated with clinical signs or symptoms.

In the post-marketing setting, cases of oligohydramnios, some associated with fatal pulmonary hypoplasia of the fetus, have been reported in pregnant women receiving trastuzumab. Therefore, trastuzumab should not be used in pregnant women. Protocols for ongoing trastuzumab studies indicate that highly effective contraceptive measures must be used.

2.3.5.3 **Summary of Activity Data for trastuzumab**

The clinical benefit of trastuzumab in women with MBC has been demonstrated in multiple clinical studies, including two pivotal studies:

A large Phase II trial (H0649g) assessed the activity of trastuzumab as a single agent in 222 women with HER2 overexpressing MBC with progressive disease after one or more chemotherapy regimens[28]. A blinded, independent response evaluation committee identified 8 complete and 26 PRs, for an ORR of 15% in the intent-to -treat population (95% CI, 11%- 21%). The median duration of response was 9.1 months, and the median duration of survival was 13 months. The most common AE, which occurred in approximately 40% of patients, were mild to moderate infusion-associated fever and/or chills. These symptoms usually occurred only during the first infusion. The most clinically significant event was cardiac dysfunction, which occurred in 4.7% of patients.

A large, open-label, randomized Phase III study (H0648g) in 469 patients with HER2-positive MBC was conducted to evaluate the efficacy of trastuzumab in combination with chemotherapy as first-line treatment[30–32]. Patients who were

anthracycline-naive were randomized to receive either anthracycline plus cyclophosphamide (AC) or trastuzumab plus AC. Patients who had received prior anthracyclines in the adjuvant setting were randomized to receive paclitaxel or trastuzumab plus paclitaxel. Patients randomized to trastuzumab and chemotherapy measurably benefited in comparison to patients treated with chemotherapy alone in terms of time to disease progression, ORR, median duration of response, and survival. As determined by an independent Response Evaluation Committee, trastuzumab prolonged median time to disease progression from 4.6 months to 7.4 months ($p<0.001$), improved the ORR (complete and PRs) from 32% to 50% ($p<0.001$), and increased median duration of response from 6.1 to 9.1 months ($p<0.001$). Compared to chemotherapy alone, the addition of trastuzumab significantly lowered the incidence of death at one year from 33% to 22% ($p=0.008$) and increased median OS 24% from 20.3 months to 25.1 months ($p=0.046$). The observed survival advantage remained despite crossover of 66% of patients initially randomized to chemotherapy alone who elected to receive trastuzumab upon disease progression. Fever/chills were observed with the initial trastuzumab infusion in approximately 25% of patients. Class III or IV cardiac dysfunction was observed in 16% of the trastuzumab + AC subgroup; increasing age was an associated risk factor for the development of cardiotoxicity in this treatment cohort.

Based on these data, trastuzumab was approved by the FDA for use in HER2-overexpressing MBC in combination with paclitaxel for first-line treatment and as a single agent for patients whose cancers progressed after prior chemotherapy for metastatic disease. However, current usage patterns of trastuzumab indicate that the drug is now being used in a broader array of circumstances that in the pivotal clinical trials. Since initiation of the pivotal clinical trials, docetaxel has become a commonly used taxane in the treatment of MBC[33] and new data have emerged on the weekly use of paclitaxel[34]. Trastuzumab has been studied in combination with paclitaxel and docetaxel using a variety of doses and schedules with promising results[35–37]. In addition, the combination of trastuzumab with vinorelbine has recently been studied[38]. In this study, 30 of 40 women treated with trastuzumab (4 mg/kg x 1, 2 mg/kg weekly thereafter) and vinorelbine (25 mg/m2 weekly, with dose adjusted each week for neutrophil count) responded to therapy, for an ORR of 75% (95% CI, 57% - 89%). Neutropenia was the only grade IV toxicity. No patients had symptomatic heart failure. Grade 2 cardiotoxicity was observed in 3 patients; prior cumulative doxorubicin dose in excess of 240 mg/m2 and borderline pre-existing cardiac function were associated with this toxicity.

Of important note for women treated on the current study is that women treated with trastuzumab-containing regimens in the past still derive benefit from pairing trastuzumab with other chemotherapy partners in the next line setting. Because of this, women often receive HER2-directed therapies beyond progression on a trastuzumab-based regimen[31,32].

2.3.6 Fulvestrant

Fulvestrant will be obtained via commercial supply. It is a current standard of care therapy for patients with ER-positive advanced breast cancer. The recommended dose of fulvestrant is 500mg by intramuscular injection on Day 1, Day 15 of cycle 1 and then on day 1 of subsequent cycles. The rationale for this dosing schedule comes from the previously presented CONFIRM trial, in which this dosing schedule was administered using either a 500mg or 250mg dose. In this trial, the 500mg dose was associated with a 4.1 month improvement in median overall survival (DiLeo et al, JNCI 2014).

Fulvestrant has previously been administered in conjunction with ribociclib with an acceptable safety profile (Tolaney et al, SABCS 2016). In a phase 1b study, fulvestrant was administered with ribociclib given either intermittently (600mg daily for 21 days, then 7 days off) or continuously (400mg daily). Common (>35%) all grade toxicities were as follows:

Common all grade toxicity	Intermittent ribociclib (n=13) (n, %)	Continuous ribociclib (n=11) (n, %)
Neutropenia	10 (77)	7 (64)
Fatigue	9 (69)	3 (27)
Nausea	6 (46)	5 (46)
Anemia	6 (46)	0 (0)
Reduced appetite	5 (39)	1 (9)

There is no anticipated interaction between the ribociclib/fulvestrant combination and trastuzumab. In Cohort C of this study, a 6 patient safety run-in phase has been incorporated to monitor for any unanticipated toxicities using the ribociclib/fulvestrant/trastuzumab triplet.

2.4 Rationale

The preclinical rationale for combining HER2-directed therapies with CDK4/6 inhibition is presented in detail in section 2.2.2. Furthermore, with the increasing use and efficacy of existing anti-HER2 therapies (trastuzumab, pertuzumab, T-DM1, lapatinib) there now exists a significant patient population who have progressed on standard therapies but retain a good performance status, and are thus good candidates for novel therapeutic combinations. Furthermore, there have been reports (in abstract form to date) of patients with advanced, treatment-refractory HER2-positive breast cancer showing partial responses to CDK4/6 inhibitors (Tolaney SABCS 2014), adding further support to the notion that the CDK4/6 pathway remains a valid target in these patients.

3. PARTICIPANT SELECTION

Laboratory tests required for eligibility must be completed within 14 days prior to the date of registration. Baseline measurements must be documented from tests within 14 days of the date of registration for protocols requiring measurable disease. Diagnostic tests, such as MRIs and CT scans, must be performed within 30 days of the date of registration.

3.1 Eligibility Criteria

3.1.1 Participants must have histologically confirmed invasive breast cancer, with locally advanced or metastatic disease. Patients without pathologic confirmation of metastatic disease should have unequivocal evidence of metastasis from physical examination or radiologic evaluation. For subjects in Cohort C, the tumor must also be hormone receptor positive, defined as demonstrating at least 1% tumor cell nuclei staining positive for either ER or PR.

3.1.2 The primary tumor, and/or metastasis must have been tested for ER, PR and HER 2, and be HER2 positive as defined by the 2013 ASCO-CAP guidelines.

3.1.3 Measurable disease by RECIST 1.1 (at least one lesion that can be accurately measured in at least one dimension > 20mm with conventional imaging techniques or > 10mm with spiral CT or MRI) or evaluable disease. Bone lesions (blastic, lytic, or mixed) in the absence of measurable disease as defined above are also acceptable.

3.1.4 Prior treatment

Cohort A:

- Prior treatment with at least one regimen containing trastuzumab and taxane.
- No prior treatment with T-DM1 that was discontinued due to disease progression or toxicity.
- No more than 4 prior lines of therapy in the metastatic setting.

Cohort B:

- Must have received prior trastuzumab, pertuzumab, and T-DM1 in neo-adjuvant, adjuvant, or metastatic setting.
- No limit on prior lines of therapies.

Cohort C:

- Must have received prior trastuzumab, pertuzumab, and T-DM1 in neo-adjuvant, adjuvant, or metastatic setting.
- Maximum of 5 prior lines of therapy for metastatic breast cancer
- Prior treatment with fulvestrant is permitted

3.1.5 Age ≥ 18 years.

3.1.6 Menopausal status

Both pre- and post-menopausal patients are permitted into the study. For patients in Cohort C who are pre-menopausal, therapy with a Gonadotropin-releasing hormone analogue (Leuprolide acetate preferred) must be commenced at least 4 weeks before commencing trial therapy. Post-menopausal status is defined either by

- Prior bilateral oophorectomy
- Age greater than 60
- Age less than 60 years with an intact uterus and amenorrhoeic for at last 12 months.
- For patients aged less than 60 years with amenorrhea for less than 12 months (including patients with prior hysterectomy, those who have received hormone replacement therapy, or those rendered amenorrhoeic by chemotherapy), follicle-stimulating hormonal (FSH) levels in the post-menopausal range define the post-menopausal state

3.1.7 ECOG performance status 0-2 (see Appendix A)

3.1.8 Participants must have adequate organ and bone marrow function as defined below:

- Absolute neutrophil count $\geq 1.5 \times 10^9/L$
- Platelets $\geq 100 \times 10^9/L$
- Hemoglobin ≥ 9 g/dL
- Total bilirubin < 1.5xULN; or total bilirubin ≤ 3.0 x ULN or direct bilirubin ≤ 1.5 x ULN in patients with well-documented Gilbert's Syndrome.
- Serum creatinine ≤ 1.5 mg/dL or calculated GFR ≥ 50mL/min
- ALT/AST <2.5x ULN; if liver metastases, ALT/AST ≤ 5.0x ULN
- INR ≤ 1.5
- Potassium, total calcium (corrected for serum albumin), magnesium, sodium and phosphorus within normal limits for the institution or corrected to within normal limits with supplements before first dose of study medication

3.1.9 Biopsies:

Cohorts B and C: all patients with disease that is deemed by the treating investigator as safely accessible to biopsy are required to undergo research biopsies as outlined in this protocol.
Cohort A: Such biopsies are optional.

3.1.10 A negative pregnancy test ≤ 7 days prior to treatment for premenopausal women and for women < 1 year after the onset of menopause.

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

3.1.12 Participants must be able to swallow ribociclib capsules or tablets.

3.1.13 Patients must have at screening a standard 12-lead ECG with mean values that meet the following parameters:

- QtcF interval at screening < 450msec (using Friderica's correction)
- Resting heart rate of 50-90bpm

3.2 Exclusion Criteria

3.2.1 Participants who have had chemotherapy within 14 days prior registration or those who have not recovered from all toxicities related to prior anticancer therapies to NCI-CTCAE version 4.03 Grade ≤1 (Exception to this criterion: patients with any grade of alopecia are allowed to enter the study). There is no washout period required for trastuzumab or for endocrine therapy; however subjects who received fulvestrant immediately prior to this trial should wait at least 28 days before receiving their first dose of fulvestrant on study

3.2.2 Participants who have received radiotherapy ≤ 2 weeks prior to starting study drug, and who have not recovered to grade 1 or better from related side effects of such therapy (except alopecia and neuropathy) and/or in whom ≥ 25% of the bone marrow was irradiated.

3.2.3 Participants who have previously received a CDK 4/6 inhibitor.

3.2.4 Participants with central nervous system (CNS) involvement unless they meet ALL of the following criteria:
- At least 4 weeks from prior therapy completion (including radiation and/or surgery) to starting the study treatment
- Clinically stable CNS tumor at the time of screening and not receiving steroids and/or enzyme-inducing anti-epileptic medications for brain metastases.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ribociclib, T-DM1 (Cohort A) and/or trastuzumab (Cohorts B and C) and/or fulvestrant (Cohort C).

3.2.6 In general, the use of any concomitant medication deemed necessary for the care of the patient is permitted in this study, except as specifically prohibited below. Combination administration of study drugs could result in drug-drug interactions (DDI) that could potentially lead to reduced activity or enhanced toxicity of the concomitant medication and/or ribociclib.

Patient is currently receiving any of the following medications and cannot discontinue use within 7 days prior to starting study drug (see (Tables 1 and 2, Appendix B for details):

- Known strong inducers or inhibitors of CYP3A4/5, including grapefruit, grapefruit hybrids, pummelos, star-fruit, and Seville oranges that have a narrow therapeutic window and are predominantly metabolized through CYP3A4/5
- Herbal preparations/medications
- Dietary supplements.

The list provided here (and in Tables 1 and 2, Appendix B) is not comprehensive and is only meant to be used as a guide. The list is based on the Oncology Clinical Pharmacology Drug-Drug Interaction Database (release date: 29 Oct 2012), which was compiled from the Indiana University School of Medicine's P450 Drug Interaction Table (http://medicine.iupui.edu/clinpharm/ddis/main-table/) and supplemented with the FDA Draft Guidance for Industry, Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling (February 2012) (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf), and the University of Washington's Drug Interaction Database (http://www.druginteractioninfo.org/). For current lists of medications that may cause QT prolongation and/or torsades de pointes (TdP), refer to the CredibleMeds® website (https://crediblemeds.org/).

Please contact the principle investigator with any questions.

3.2.7 Participants who have any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, cause unacceptable safety risks, contraindicate patient participation in the clinical trial or compromise compliance with the protocol (e.g. chronic pancreatitis, chronic active hepatitis, active untreated or uncontrolled fungal, bacterial or viral infections, etc.).

3.2.8 Participants who have had major surgery within 2 weeks prior to starting study drug or has not recovered from major side effects (tumor biopsy is not considered as major surgery).

3.2.9 Participants who have clinically significant, uncontrolled heart disease and/or cardiac repolarization abnormalities including any of the following:

- History of acute coronary syndromes (including myocardial infarction, unstable angina, coronary artery bypass grafting, coronary angioplasty, or stenting) or symptomatic pericarditis within 6 months prior to screening
- History of documented congestive heart failure (New York Heart Association functional classification III-IV)
- Clinically significant cardiac arrhythmias (e.g. ventricular tachycardia), complete left bundle branch block, high-grade AV block (e.g. bifascicular block, Mobitz type II and third-degree AV block
- Documented cardiomyopathy
- Left Ventricular Ejection Fraction (LVEF) < 50% as determined by Multiple Gated acquisition (MUGA) scan or echocardiogram (ECHO) at screening
- Long QT syndrome or family history of idiopathic sudden death or congenital long QT syndrome, or any of the following
    - Risk factors for Torsades de Pointe (TdP) including uncorrected hypokalemia or hypomagnesemia, history of cardiac failure, or history of clinically significant/symptomatic bradycardia.
    - Inability to determine the QT interval on screening (QTcF, using Fridericia's correction)
- Systolic blood pressure (SBP)>160 mmHg or <90 mmHg at screening

3.2.10 Known history of HIV-positivity.

3.2.11 Active Hepatitis B and/or Hepatitis C Infection

3.2.12 Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of the study drugs (e.g., uncontrolled ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection).

3.2.13 Concurrent malignancy or a malignancy within 3 years prior to starting study drug, with the exception of adequately treated basal or squamous cell carcinoma, non-melanomatous skin cancer or curatively resected cervical cancer. Participants with malignancies less than 3 years prior to registration may be considered eligible after discussion with the principle investigator.

3.2.14 Participants who are currently receiving or have received systemic corticosteroids ≤2 weeks prior to starting study drug, or who have not fully recovered from side effects of such treatment. The following uses of corticosteroids are permitted: single doses, topical applications (e.g., for rash), inhaled sprays (e.g., for obstructive airways diseases), eye drops or local injections (e.g., intra-articular).

3.2.15 Patient is currently receiving warfarin or other coumarin-derived anticoagulant for treatment, prophylaxis or otherwise. Therapy with heparin, low molecular weight heparin (LMWH) or fondaparinux is allowed.

3.2.16 Participation in a prior investigational study within 21 days prior to enrollment or within 5 half-lives of the investigational product, whichever is shorter.

3.2.17 Patient with a Child-Pugh score B or C.

3.2.18 Participants who have a history of non-compliance to medical therapies.

3.2.19 Pregnant women are excluded from this study because ribociclib has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ribociclib, breastfeeding should be discontinued if the mother is treated with ribociclib. These risks also apply to trasutuzumab used in this study. Pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive urine or hCG laboratory test (>5 mIU/mL).

3.2.20 Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception throughout the study and for 8 weeks after study drug discontinuation. Highly effective contraception methods include:

- Total abstinence when this is in line with the preferred and usual lifestyle of the patient.
- Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male sterilization (at least 6 months prior to screening). For female patients on the study, the vasectomized male partner should be the sole partner for that patient
- Combination of the two following
    a. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
    b. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository.

Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

3.2.21 Sexually active males unless they use a condom during intercourse while taking the drug and for 4 months after stopping treatment and should not father a child in this period. A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following

registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

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

5. TREATMENT AND/OR IMAGING PLAN

5.1 Treatment Regimen

Treatment will be administered on an outpatient basis. Reportable adverse events and potential risks and reporting guidelines are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Dosing Schedule for Cohort A			
Dose Level	Ribociclib	T-DM1	Cycle Length
-1	200mg[b] PO daily	3.6 mg/kg IV every 21 days	21 days
1[a]	300mg[b] PO daily	3.6 mg/kg IV every 21 days	
2	400mg[b] PO daily	3.6 mg/kg IV every 21 days	
3	500mg[b] PO daily	3.6 mg/kg IV every 21 days	
4	600mg[b] PO daily	3.6 mg/kg IV every 21 days	
a. starting dose level b. ribociclib will be given for two weeks (day 8-21) of a 21 day cycle			

Dosing Schedule for Cohort B, Phase 1b				
Dose Level	Ribociclib		Trastuzumab	Cycle Length
Level 0[a]	400mg PO daily	2x 200 mg capsules or tablets	8mg/kg IV Loading dose[b, c] 6mg/kg IV[b, c] every 21 days	21 days
Level -1	300mg PO daily	1x 200 mg + 2x 50 mg capsules or tablets		
Level -2	300mg PO days 1-14	1x 200 mg + 2x 50 mg capsules or tablets		
a. starting dose level b. Load of trastuzumab is NOT required if trastuzumab 6mg/kg dose was administered within 28 days of protocol start or if 2mg/kg dose administered within 2 weeks of protocol start. c. First infusion trastuzumab should be given over 90min with a 60min post-infusion observation. Subsequent infusions should be given over 30-60min as tolerated with a 30min post-infusion observation.				

Dosing Schedule for Cohort B, Phase II			
Ribociclib		Trastuzumab	Cycle Length
Dose determined by Phase Ib	PO capsules or tablets	8mg/kg IV Loading dose[b, c]: 6mg/kg IV[b, c] every 21 days	21 days
a. starting dose level b. Load of trastuzumab is NOT required if trastuzumab 6mg/kg dose was administered within 28 days of protocol start or if 2mg/kg dose administered within 2 weeks of protocol start. c. First infusion trastuzumab should be given over 90min with a 60min post-infusion observation. Subsequent infusions should be given over 30-60min as tolerated with a 30min post-infusion observation.			

Dosing Schedule for Cohort C, Phase 1b					
Dose Level	Ribociclib		Trastuzumab	Fulvestrant	Cycle Length
Level 0[a]	400mg PO daily	2x 200mg capsules or tablets	6 mg/kg IV loading dose[b,c] 4mg/kg IV every 14 days[b,c]	500mg IM every 14 days for 29 days, then 500mg IM every 28 days[d]	28 days
Level -1	400mg PO days 1-21	2x 200mg			
Level -2	300mg PO daily	1x 200mg + 2x 50mg capsules or tablets			
Level -3	200mg PO daily	1x 200mg capsule or tablet			
a. Starting dose level b. Load of trastuzumab is NOT required if trastuzumab 6mg/kg was administered within 28 days of protocol start or if 2mg/kg dose administered within 2 weeks of protocol start. c. First infusion of trastuzumab should be given over 90 minutes with a 60min post-infusion observation. Subsequent infusions should be given over 30-60min as tolerated with a 30min post-infusion observation. d. C1D15 dose of fulvestrant should not be given in the subject has received fulvestrant within 6 months of study start.					

Dosing Schedule for Cohort C, Phase II				
Ribociclib		Trastuzumab	Fulvestrant	Cycle Length
Dose determined by Phase Ib	PO capsules or tablets	6 mg/kg IV loading dose[a,b] 4mg/kg IV every 14 days[a,b]	500mg IM every 14 days for 29 days, then 500mg IM every 28 days[c]	28 days

a. Load of trastuzumab is NOT required if trastuzumab 6mg/kg was administered within 28 days of protocol start or if 2mg/kg dose administered within 2 weeks of protocol start.
b. First infusion of trastuzumab should be given over 90 minutes with a 60min post-infusion observation. Subsequent infusions should be given over 30-60min as tolerated with a 30min post-infusion observation.
c. C1D15 dose of fulvestrant should not be given in the subject has received fulvestrant within 6 months of study start.

Treatment Information Summary

Treatment Arm	# of Pts Planned	Type of Study Drug	Compound	Min Dose and unit	Max Dose and unit	Frequency	Route
Cohort A: Dose-escalation	15-30	CDK 4/6 inhibitor	Ribociclib	200mg	600mg	Day 8-21	PO
		Anti-HER2 therapy	T-DM1	2.4mg/kg	3.6 mg/kg	Every 21 days	IV
Cohort A: Dose-expansion	15	CDK 4/6 inhibitor	Ribociclib	200mg	600mg	Day 8-21	PO
		Anti-HER2 therapy	T-DM1	2.4 mg/kg	3.6 mg/kg	Every 21 days	IV
Cohort B: Safety run-in	6	CDK 4/6 inhibitor	Ribociclib	300mg	400mg	Max: 400mg continuous. Min: 300mg Day 1-14	PO
		Anti-HER2 therapy	Trastuzumab	8mg/kg loading dose then 6 mg/kg[a]		Every 3 weeks	IV
Cohort B: Main cohort	35	CDK 4/6 inhibitor	Ribociclib	300mg	400mg	Continuous	PO
		Anti-HER2 therapy	Trastuzumab	8mg/kg loading dose then 6 mg/kg[a]		Every 3 weeks	IV
Cohort C: Safety Run-In	6	CDK 4/6 Inhibitor	Ribociclib	300mg	400mg	Max: 400mg continuous, Min: 300mg Day 1-14	PO
		Anti-HER2 Therapy	Trastuzumab	6mg/kg loading dose then 4mg/kg[a]		Every 14 days	IV
		Hormonal Therapy	Fulvestrant	500mg every 14 days for 29 days; then 500mg every 28 days[b]		Every 28 days	IM
Cohort C: Main Cohort	30 or 36	CDK 4/6 Inhibitor	Ribociclib	300mg	400mg	Max: 400mg continuous, Min: 300mg Day 1-14	PO
		Anti-HER2 Therapy	Trastuzumab	6mg/kg loading dose then 4mg/kg[a]		Every 14 days	IV
		Hormonal Therapy	Fulvestrant	500mg every 14 days for 29 days; then 500mg every 28 days[b]		Every 28 days	IM

a. Load of trastuzumab is NOT required if trastuzumab 6mg/kg dose was administered within 28 days of protocol start or if 2mg/kg dose administered within 2 weeks of protocol start.
b. Day 15 dose of fulvestrant is not required in the first cycle if the patient received prior fulvestrant within 6 months of starting protocol therapy.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

At every visit, physical examination and laboratory tests will be performed. Labs do not need to re-meet eligibility criteria.

Pre-treatment criteria will include:

- absolute neutrophil count (ANC) $\geq$1,500/m^3
- platelets $\geq$50,000/mm^3
- Hemoglobin $\geq$ 9 g/dL
- Total Bilirubin $\leq$ 1.5 times institutional upper limit of normal (ULN)
- Serum creatinine $\leq$ 1.5 mg/dL OR calculated GFR $\geq$ 50mL/min
- ALT/AST $\leq$ 2.5x ULN; if liver metastases, ALT/AST $\leq$5.0x ULN
- QT interval of $\leq$ 470ms.
- LVEF $\geq$ 50%.

5.2.2 Subsequent Cycles

- ANC must be $\geq$ 1,000/m^3
- Platelets $\geq$50,000/mm^3
- All non-hematologic toxicities, related to study drug, must be $\leq$ grade 1 or returned to baseline

5.3 Agent Administration

5.3.1 Ribociclib

- Ribociclib will be taken orally, once a day.
  Cohort A: for two weeks (day 8-21) of a 21 day cycle
  Cohort B: continuously for a 21-day cycle of treatment (except at Dose Level -2, when ribociclib is given Days 1-14 of a 21 day cycle)
  Cohort C: Dose level 0 for ribociclib in phase 1b component is 400mg daily for Days 1-28 of a 28 day cycle. Dose level -1 in this cohort is 400mg daily for Days 1-21 of a 28 Day cycle. Starting dose for phase 2 component will be determined from phase 1b patients. Dose reductions thereafter are outlined in Tables 6-3 and 6-4.
- Ribociclib will be dosed on a flat dosing scale of mg/day, irrespective of body size and weight.
- Participants must be instructed to take their once-a-day dose with a large glass of water (~250ml). Ribociclib can be taken at any time of day but should be taken at approximately the same time each day.
- Ribociclib can be taken without regard to meals; however dietary habits around the time of dosing should be as consistent as possible throughout the study [and in particular during those periods when samples are being taken for PK analysis].
- Participants should be instructed to swallow the ribociclib capsules or tablets whole and not to chew, crush or open them.
- If vomiting occurs during the course of the treatment, no re-dosing of the patient is allowed before the next scheduled dose.
- Any doses that are missed (not taken within 6 hours of the intended time) should be skipped and should not be replaced or made up on a subsequent day.
- Participants should inform the investigational site staff of any missed, vomited or delayed doses.
- Patients must avoid consumption of grapefruit grapefruit hybrids, pummelos, star-fruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medication, due to potential CYP3A4 interaction with the study medications. Orange juice is allowed.
- No herbal or dietary supplements are permitted, due to potential interactions with ribociclib; multivitamins are allowed.

- Tablets will be dispensed once the capsule supplies are exhausted.
- Please dispense a precise count of Ribociclib (LEE011) tablets to patients.

On days with PK, ECG sampling, chemistry panel and/or lipid panel sampling, the following additional guidelines should be followed:

- On a day when PK blood collection is scheduled at the clinic, patients must take study treatment in the clinic under the supervision of the Investigator or designee. On all other days patients may take the study treatment at home.
- On a day of lipid panel sampling, patients must be fasting from all food and drink for at least 8 hours overnight. Water is allowed during all fasting periods; however coffee, tea and juice are not permitted during the fasting period. Patients must also take study treatment in the clinic under the supervision of the Investigator or designee. On all other days patients may take the study treatment at home.
- Pre-dose samples should be drawn prior to dosing. The sampling time of the PK samples and the dosing time must be precisely recorded in the CRF. Furthermore, the dosing date and time the study medication was taken on the day before the PK assessment must be precisely recorded in the CRF. Post-dose PK samples should be collected after dosing of the study treatment.

5.4 Agent Administration

5.4.1 T-DM1 and trastuzumab

Administration
T-DM1 and trastuzumab will be administered as per routine standard of practice. Details in package inserts (available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf) and http://www.accessdata.fda.gov/drugsatfda_docs/label/2000/trasgen020900lb.htm) are summarized below.

Both agents will be given as IV infusions and should not administered as either an IV push or bolus. The first infusion of T-DM1 will be administered over 90 minutes (± 10 minutes). Infusions may be slowed or interrupted for patients experiencing infusion-associated symptoms. Vital signs must be assessed before and after dose administration in the first infusion. Trastuzumab will also be administered as an IV infusion – the first dose will be administered over 90 minutes, and subsequent maintenance doses will be given over 30-60 minutes. If a patient has not received a dose of trastuzumab within 28 days of starting cohort B, the initial dose of trastuzumab will be 8mg/Kg over 90 minutes. Maintenance dose of trastuzumab is 6mg/Kg over 30-60 minutes.

Dosing
T-DM1 will be given as intravenous infusion on Day 1 of a 3-week cycle at a dose of 3.6 mg/kg IV. Body weight will be recorded at the screening visit and used for dosing. Weights should be checked day 1 of each cycle of treatment. If there is a greater than 5% change in weight or BSA, the dose must be recalculated.
Dose delays and modifications for specific T-DM1 related toxicities are described in Table 6-7 and 6-8. Dose delays of up to 42 days from last dose are permitted. For delayed or missed doses of trastuzumab, if the time between 2 sequential infusions is less than 6 weeks, the 6mg/Kg IV dose of trastuzumab should be administered. Do not wait until the next planned dose. If the time between 2 sequential infusion is 28 days or more, the initial dose of 8mg/Kg over 60 minutes should be re-administered followed every 3 weeks thereafter by a dose 6mg/Kg administered over 30-60 minutes.

Observation period
Following the initial dose of T-DM1, patients will be observed for at least 90 minutes for fever, chills, or other infusion-associated symptoms. If prior infusions were well tolerated (without any signs or symptoms of infusion reactions), subsequent doses of T-DM1 may be administered over 30-90 minutes (± 10 minutes), with a minimum 30-minute observation period after infusion. Local health authority guidelines must be followed with regard to further observation and monitoring, if applicable. For trastuzumab, participants should be observed for fever and chills or other infusion-associated symptoms for 60 minutes for the first infusion and 30 minutes for subsequent infusions.

Infusion Reactions
Premedication with steroids or antihistamines to prevent hypersensitivity reactions is not required with the use of trastuzumab or T-DM1. Of note, as treatment with T-DM1 has not been studied in patients who had trastuzumab permanently discontinued due to infusion-related reactions (IRR) and/or hypersensitivity, treatment with T-DM1 is not recommended for these patients.

Observation periods following T-DM1 or trastuzumab infusion are described above. If a significant infusion associated reaction (IAR) occurs, the infusion should be interrupted and appropriate medical therapies should be administered (see below). Permanent discontinuation should be considered in patients with severe IAR. This clinical assessment should be based on the severity of the preceding reaction and response to administered treatment for the adverse reaction.

If patients develop an IAR, patients should be treated according to the following guidelines, or according to institutional guidelines, at discretion of the study physician:

- Stop infusion and notify physician.
- Assess vital signs.
- Administer acetaminophen 650mg PO.
- Consider administration of:
  - Meperidine 50 mg IM,
  - Diphenhydramine 50mg IV,
  - Ranitidine 50mg IV or cimetidine 300mg IV,
  - Dexamethasone 10mg IV or famotidine 20mg IV.
- If vital signs stable, resume trastuzumab infusion.
- Patients tend not to develop infusion syndromes with subsequent cycles. No standard premedication is required for future treatments if patients have developed an infusion syndrome. Patients may be given acetaminophen prior to treatments.

Serious Infusion-Associated Events.
Serious reactions have been treated with supportive therapy such as oxygen, beta-agonists, corticosteroids and withdrawal of study agent as indicated.

These guidelines should never replace sound clinical judgment.

5.4.2 Fulvestrant

Fulvestrant is available commercially, should be stored and supplied according to the labels, and will not be supplied by the study. It should be injected into the buttock slowly (1-2 minutes per injection) as two 250mg injections, one in each buttock. However, for patients with moderate hepatic impairment (defined as Childs-Pugh Class B), including any patient who develops moderate hepatic impairment during the study treatment, fulvestrant 250mg IM should be administered into the buttock slowly (1-2 minutes) as one 250mg injection. Subjects who have received prior fulvestrant within 6 months of initiating protocol therapy should not be given the D15 dose in the first cycle.

5.5 Definition of Dose-Limiting Toxicity (DLT)

A DLT is defined as an AE or clinically significant abnormal laboratory value assessed as having a reasonably possible relationship to the study medication(s) and is unrelated to disease, disease progression, inter-current illness, or concomitant medications that occurs within the first cycle (21 days) of treatment with ribociclib in combination with either T-DM1 (Cohort A) or trastuzumab (Cohort B) and meets any of the criteria included in the table below. NCI CTCAE version 4.03 should be used for all grading.

Whenever a patient experiences toxicity that fulfills the criteria for a DLT, treatment with the study drug combination will be interrupted and the toxicity will be followed up. For the purposes of dose escalation and determination of MTD, DLTs that occur during the first cycle will be necessarily considered, including those in which the event started in Cycle 1 and the confirmation of the DLT occurs in a subsequent cycle. The investigator must notify the Sponsor immediately of any unexpected CTCAE grade > 3 adverse events or laboratory abnormalities. Prior to enrolling patients into a higher dose level, CTCAE grade > 2 adverse events will be reviewed for all patients at the current dose level.

Appropriate eligibility criteria and specific DLT definitions, as well as specific dose modification and stopping rules are included in this protocol. Recommended guidelines for prophylactic or supportive treatment for expected toxicities, including management of study drug induced adverse events are provided in Section 6.

For patients who do not tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study treatment. These changes must be recorded on the Dosage Administration Record eCRF.

Cohort A

Toxicity	**DLT Criteria for Cohort A**
Hematology	CTCAE grade 3 thrombocytopenia with bleeding
	CTCAE grade 3 or 4 febrile neutropenia
	CTCAE grade 4 neutropenia lasting more than 7 consecutive days
	CTCAE grade 4 thrombocytopenia
	CTCAE grade 4 lymphopenia lasting more than 7 consecutive days
ECG QT interval	QTc interval ≥ 501 ms on at least 2 separate ECGs
Cardiac	Cardiac toxicity ≥ CTCAE grade 3 Clinical signs of cardiac disease, such as unstable angina or myocardial infarction, or Troponin ≥ CTCAE grade 3
Gastro-intestinal	≥ CTCAE grade 3 nausea or vomiting ≥ 48 hrs despite optimal anti-emetic therapy
	≥ CTCAE grade 3 diarrhea ≥ 48 hrs despite optimal anti-diarrhea treatment
Hepato-biliary	≥ CTCAE grade 2 total bilirubin for more than 7 consecutive days
	≥ CTCAE grade 3 total bilirubin
	CTCAE grade 2 ALT with a ≥grade 2 bilirubin elevation of any duration in the absence of liver metastases
	≥ CTCAE grade 3 ALT for >4 consecutive days
	CTCAE grade 4 ALT or AST
	Grade 4 serum alkaline phosphatase >7 consecutive days
Renal	≥ CTCAE grade 3 serum creatinine
Events not described above	≥ CTCAE grade 3, except for the exclusions noted below
	rash >v48 hours despite adequate treatment.
Exceptions to DLT criteria	Alopecia of any grade
	< 5 days of CTCAE grade 3 fatigue
	≤48 hours of CTCAE grade 3 edema
	Grade 3 laboratory abnormalities that are responsive to oral supplementation or deemed by the investigator to be clinically insignificant
CTCAE version 4.03 should be used for grading. Optimal therapy for vomiting and diarrhea should be based on institutional guidelines with consideration of the prohibited medications listed in these protocol guidelines.	

Cohorts B and C

Toxicity	DLT Criteria for Cohort B and C
Hematology	CTCAE grade 4 neutropenia lasting more than 7 consecutive days
	CTCAE grade 4 thrombocytopenia
	CTCAE grade 3 thrombocytopenia with bleeding
	CTCAE grade 3 or 4 febrile neutropenia
ECG QT interval	QTc interval ≥ 501 ms on at least 2 separate ECGs
Cardiac	Cardiac toxicity ≥ CTCAE grade 3 Clinical signs of cardiac disease, such as unstable angina or myocardial infarction, or Troponin ≥ CTCAE grade 3
Gastro-intestinal	≥ CTCAE grade 3 vomiting ≥48 hours despite optimal anti-emetic therapy ≥ CTCAE grade 3 diarrhea ≥48 hours despite optimal anti-diarrhea treatment
Hepato-biliary	≥ CTCAE grade 2 total bilirubin for more than 7 consecutive days
	≥ CTCAE grade 3 total bilirubin
	CTCAE grade 2 ALT with a ≥grade 2 bilirubin elevation of any duration in the absence of liver metastases
	≥ CTCAE grade 3 ALT for >4 consecutive days
	CTCAE grade 4 ALT or AST
	Grade 4 serum alkaline phosphatase >7 consecutive days
Renal	≥ CTCAE grade ≥3 serum creatinine
Non-hematologic events	≥ CTCAE grade 3, except for the exclusions noted below
Exceptions to DLT criteria	Alopecia of any grade
	< 5 days of CTCAE grade 3 fatigue
	Grade 3 fever or infection without neutropenia < 5 days duration
	Grade 3 laboratory abnormalities that are responsive to oral supplementation or deemed by the investigator to be clinically insignificant
CTCAE version 4.03 should be used for grading. Optimal therapy for vomiting and diarrhea should be based on institutional guidelines with consideration of the prohibited medications listed in these protocol guidelines.	

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.6 General Concomitant Medication and Supportive Care Guidelines

Administration of concomitant medication during the DLT period may require the subject to be replaced. Decisions regarding replacements of subjects requiring concomitant medication will be discussed with the sponsor on a case-by-case basis.

5.6.1 Permitted concomitant medication

Medications required to treat adverse events, manage cancer symptoms, concurrent diseases and supportive care agents, such as pain medications, anti-emetics and anti-diarrheal agents are allowed.

The patient must be told to notify the investigational site about any new medications he/she takes consenting to study treatment. All medications (other than study drugs) and significant non-drug therapies (including vitamins, herbal medications, physical therapy and blood transfusions) administered within 30 days of study entry and during the study must be listed on the Concomitant medications/Significant non-drug therapies section of the patient record.

Patients taking concomitant medication chronically should be maintained on the same dose and dose schedule throughout the study period, as medically feasible.

Treatment with bisphosphonates and denosumab are permitted.

Use of hematopoietic growth factors is not allowed during the DLT period.

Palliative radiation may be permitted if done solely for bone pain relief after discussion and approval from the principal investigator. It should not be delivered to a target lesion and it should not encompass more than 25% of irradiated bone marrow. If palliative radiotherapy is initiated after start of study treatment, the reason for its use must be clearly documented and progression as per RECIST 1.1 must be ruled out. Treatment with ribociclib should be held during palliative radiation therapy.

CNS radiation may be permitted if new CNS metastases are discovered, or if progressive disease in the CNS occurs, in the absence of systemic progression, if it is amenable to radiation and/or surgery if treatment is indicated. Ribocicib and T-DM1 should be held for 7 days before, during, and 7 days after CNS radiation or surgery. Treatment with trastuzumab can be continued through radiation. If CNS disease is treated, in order to continue on study, the study treatment must be resumed within 42 days of the last dose. At the time of CNS progression, the patient must have an ECOG performance status of 0-2. If study treatment is resumed, these patients will be allowed to continue to receive therapy.

5.6.2 Permitted concomitant therapy requiring caution

Medications to be used with caution during ribociclib and T-DM1 or trastuzumab in this study are listed below. This list is not comprehensive and is only meant to be used as a guide. These medications should be excluded from patient use if possible. If they must be given, then use with caution and consider a ribociclib interruption if the concomitant medication is only needed for a short time. (see Table 1 in Appendix B).

- Moderate inhibitors or inducers of CYP3A4/5
- Sensitive substrates of CYP3A4/5 that do not have a narrow therapeutic index
- Strong inhibitors of BSEP
- Sensitive substrates of the renal transporters, MATE1 and OCT2
- Sensitive substrates of BCRP
- Medications that carry a possible risk for QT prolongation

5.6.3 Prohibited concomitant therapy

The following medications are prohibited during study treatment in the study (see Table 2 in Appendix B). This list is not comprehensive and is only meant to be used as a guide.

- Strong inhibitors or inducers of CYP3A4/5
- Substrates of CYP3A4/5 with a narrow therapeutic index
- Other investigational and antineoplastic therapies not part of the study
- Herbal medications/preparations, dietary supplements (except for vitamins). Herbal medications include, but are not limited to: St. John's wort, Kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, black cohosh and ginseng. Patients should stop using all herbal medications and dietary supplements at least 7 days prior to first dose of study treatment.

- Radiation therapy (except for palliative radiotherapy at focal sites) is not permitted. However individual exceptions to this can be discussed with the principal investigator in cases where radiation is thought to be clinically warranted, such as for brain metastases, in the absence of systemic disease progression. In all cases, there must remain non-irradiated sites of disease, measurable disease accessible by RECIST 1.1.
- Cohort A: Strong inhibitor or inducers of CYP2C8. Known strong inhibitors of CYP2C8 include, but are not limited to gemfibrozil, montelukast, quercetin, and rosiglitazone. Known strong inducers of CYP2C8 include, but are not limited to rifampin. These are only partial lists. For additional information including drug elimination half-lives of strong inhibitors and inducer (please see Table 3 in Appendix B)
- The weak inhibitory effect on cytochrome P450 enzymes exhibited by trastuzumab in vitro suggests a low risk of interaction with other drugs coadministered in usual clinic practice.

5.6.4 Supportive care guidelines

Anti-emetics
Participants may be given anti-emetics at the discretion of the treating physician. Because of the low emetogenic potential of ribociclib, T-DM1 and trastuzumab, the following antiemetics are recommended if the patient experiences symptoms:

- Prochlorperazine 10mg IV/PO x1 PRN, and/or
- Lorazepam 1 mg IV/PO x 1 PRN.

These guidelines should never replace sound clinical judgment.

Anti-Diarrheal therapy
To prevent dehydration, early treatment of diarrhea with anti-diarrheal medication should be considered and patients treated with fluids and electrolyte replacement, as clinically indicated. Patients should be treated according to the following guidelines, or according to institutional guidelines, at the discretion of the study physician:

For uncomplicated grade 1-2 diarrhea:

- Stop all lactose containing products
- Drink 8-10 large glasses of clear liquids a day
- Eat frequent small meals
- Administer standard dose of loperamide: Initial dose 4mg followed by 2mg every 4 hours or after every unformed stool, up to 16mg per day. Continuation of loperamide suggested until diarrhea free for 12 hours

For grade 3 or 4 diarrhea or grade 1-2 with complicating features (severe cramping, severe nausea or vomiting, decreased performance status, fever, sepsis, grade 3 or 4 neutropenia, frank bleeding, dehydration):

- Use intravenous fluids as appropriate, consider hospital admission;
- Use prophylactic antibiotics as needed (e.g. fluoroquinolones), especially if diarrhea is persistent beyond 24 hours or there is fever or grade 3-4 neutropenia;

These guidelines should never replace sound clinical judgment.

Management of peripheral neuropathy guidelines
Early recognition and subsequent treatment delay or dose reduction can improve symptoms in most cases. Assessment for peripheral neuropathy should be done at every visit. Data are insufficient to support the routine use of any agent as prophylaxis or for treatment.

Management of Hematologic Toxicities

Care should be taken to carefully monitor the patient's hematologic status throughout the course of the trial.

Febrile neutropenia
All febrile neutropenic patients should have a comprehensive clinical history and physical examination, as well as laboratory, microbiology, and imaging evaluation at the discretion of the treating physician. Patients with febrile neutropenia should be treated immediately with empiric broad-spectrum antibiotics at choice of the treating physicians. Initial antibiotic selection should be guided by the patient's history, particularly hypersensitivity, risk category and recent antibiotic use, culture data, and institutional nosocomial infection patterns. Antibiotics used in this setting should be bactericidal.

High risk febrile neutropenia includes the following categories: absolute neutrophil count ≤ 100cells/microL anticipated to last more than 7 days, age >65 year-old, uncontrolled primary disease, pneumonia, hypotension, multiorgan dysfunction (sepsis syndrome), invasive fungal infection, being hospitalized at the time of the development of fever.

In addition to antibiotics, central venous catheter removal is recommended for patients with catheter-related bloodstream infections in which any of the following organisms are implicated: *Staphylococcus aureus, Pseudomonas aeruginosa, fungi* (eg, *Candida spp*), or rapidly-growing nontuberculous mycobacteria, as well as for patients with complicated infections (eg, tunnel infection, port pocket infection).

If an infectious source of fever is identified, antibiotics should be continued for at least the recommended duration for that particular pathogen and site of infection and until resolution of fever and neutropenia. If the infectious source is not identified the duration of the treatment will dependent on the resolution of the fever and neutropenia.

These guidelines should never replace sound clinical judgment.

Management of Cardiac Safety

All participants must have a baseline evaluation of cardiac function including a measurement of LVEF by either MUGA or ECHO prior to entry into the study. Only participants with an LVEF ≥ 50% should be entered into this study.

All participants should have cardiac monitoring during treatment with trastuzumab or T-DM1. Echo or MUGA scans should be scheduled at the same radiology facility where the participant's baseline ECHO or MUGA was conducted. LVEF measurements are required at baseline and at least every 4 cycles.

During the course of trastuzumab therapy, participants should be monitored for signs and symptoms of CHF (i.e., dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain). The confirmation of the CHF diagnosis should include the same method used to measure at baseline (either ECHO or MUGA).

Trastuzumab and Trastuzumab-DM1 should be discontinued in any participant who develops clinical signs and symptoms suggesting CHF. CHF should be treated and monitored according to standard medical practice. At present; there are inadequate data available to assess the prognostic significance of asymptomatic drops in LVEF.

Refer to Appendix C for Algorithm for Continuation and Discontinuation of HER2 Targeted Study medication. If LVEF is <40% or is 40-50% with a 10% or greater absolute decrease below the pretreatment value, withhold trastuzumab and repeat LVEF assessment within approximately 3 weeks. If after a repeat assessment, the LEVF has not improved, or has declined

further, trastuzumab will be discontinued.

Refer to Appendix D to New York Heart Association Cardiac disease Classification.

5.7 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for *[# cycles]* or until one of the following criteria applies:

- Disease progression
- If ribociclib is held for any reason for 42 days or longer, unless exception made by the PI for clinical benefit
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

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

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

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Sara Tolaney at 617-632-2335.

5.8 Duration of Follow Up

5.8.1 Safety follow-up

After discontinuation of study treatment, all participants will be followed for safety for at least 30 days except in case of death, loss to follow up or withdrawal of consent.

5.8.2 Efficacy follow-up

Participants who discontinue treatment for reasons other than disease progression will continue to be followed every 8 weeks for efficacy (i.e., tumor assessments and participants reported outcomes) during the first 18 months and every 12 weeks thereafter until disease progression, death, withdrawal of consent, loss to follow-up, subject/guardian decision or until the start of a new antineoplastic treatment.

5.8.3 Survival follow-up

All participants will be followed for survival once they discontinue study treatment and tumor evaluations until the study is stopped. Survival follow-up will be done every 12 weeks or earlier if a survival update is required to meet safety needs. Survival information can be obtained by clinical visits or telephone calls until death, the participant is lost to follow up, or the participant withdraws consent for survival follow-up.

5.9 Criteria for Taking a Participant Off Study

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

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

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

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

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

For participants who do not tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study treatment. These changes must be recorded on the Dosage Administration Record CRF.

All AEs and clinically significant laboratory abnormalities should be assessed by the Investigator for relationship to study drug. In the event that the relationship is unclear, discussion should be held with the study principal investigator, to discuss which study drug(s) should be held and/or modified. An AE may be considered related to Anti-HER2 drug alone, ribociclib alone, to both drugs, or to neither. Dosing should be modified (including holding the dose, dose reduction, or discontinuation of drug) as described below.

Dose reductions or treatment interruption for reasons other than those described below may be made by the Investigator if it is deemed in the best interest of patient safety. Once reduced the dose may be re-escalated after 2 cycles on a stable dose without drug interruption and after discussion with the principal investigator.

6.1 Ribociclib

6.1.1 Dose modification guideline

Management of severe or intolerable adverse reactions requires temporary dose reduction and/or interruption of ribociclib therapy. For patients who do not tolerate the dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study treatment.

Table 6-1: Dose modification of Ribociclib for Cohort A

Dose Level	Ribociclib	T-DM1	Cycle Length
Starting dose	300mg PO, Day 8-21	3.6 mg/kg IV q3w	21 days
First dose reduction	250mg PO, Day 8-21		
Second dose reduction	200mg PO, Day 8-21		

Table 6-2: Dose modification of Ribociclib for Cohort B

Dose Level	Ribociclib	Trastuzumab	Cycle Length
Starting dose	400mg PO, continuous daily[a]	8mg/Kg loading dose 6mg/Kg q3w subsequent infusions	21 days
First dose reduction	300mg PO, continuous daily		
Second dose reduction	300mg PO, Day 1-14		
a. The starting dose of ribociclib in phase II will be defined based on the phase Ib findings.			

Recommendations for dose reduction, interruption or discontinuation of ribociclib in the management of adverse reactions are summarized below. Clinical judgment of the treating physician should guide the management plan of each patient based on individual benefit/risk assessment.

Table 6-3: Dose modification of Ribociclib for Cohort C Phase Ib and Phase II (if dose established from safety run-in is 400mg daily[a])

Dose Level	Ribociclib	Trastuzumab	Fulvestrant	Cycle Length
Starting dose	400mg PO, continuous daily	6mg/Kg loading dose 4mg/Kg q2w subsequent infusions	500mg every 14 days for 29 days, then 500mg every 28 days	28 days
First dose reduction	400mg PO daily for Days 1-21 of 28 day cycle			
Second dose reduction	300mg PO, continuous daily			
Third dose reduction	200mg PO, continuous daily			
a. The starting dose of ribociclib in Phase II will be define based on the phase Ib findings				

Table 6-4: Dose Modifications of Ribociclib for Cohort C Phase II (if dose established from safety run-in is 400mg PO daily for Days 1-21 of 28 day cycle[a])

Dose Level	Ribociclib	Trastuzumab	Fulvestrant	Cycle Length
Starting dose	400mg PO daily for Days 1-21 of 28 day cycle	6mg/Kg loading dose 4mg/Kg q2w subsequent infusions	500mg every 14 days for 29 days, then 500mg every 28 days	28 days
First dose reduction	300mg PO, continuous daily			
Second dose reduction	200mg PO, continuous daily			
a. The starting dose of ribociclib in Phase II will be define based on the phase Ib findings				

6.1.2 Hematological toxicities

Table 6-5: Ribociclib dose adjustment and management recommendation for hematological adverse reactions possibly related to study drug

Toxicity	Grade	Dose Adjustment and Management Recommendations
Thrombocytopenia	1 ≥75 x 10^9/L	No dose adjustment required.
	2 ≥50 x 10^9/L - <75 x 10^9/L	Dose interruption until recovery to grade ≤1. Restart ribociclib at the same dose.
	3 ≥25 x 10^9/L - <50 x 10^9/L	Dose interruption until recovery to grade ≤1. Restart ribociclib at the same dose level. • If toxicity recurs at grade 3 within next two cycles (42 days): temporary dose interruption until recovery to grade ≤1 and reduce ribociclib to the next lower dose level.
	4 <25 x 10^9/L	Dose interruption until recovery to grade ≤1. Re-initiate ribociclib at the next lower dose level. • If toxicity recurs at grade 4 within next two cycles (42 days): discontinue ribociclib.
Absolute neutrophil count (ANC)	1 ≥1.5 x 10^9/L	No dose adjustment required.
	2 ≥1.0 - <1.5 x 10^9/L	No dose adjustment required.

	3
	≥0.5 - <1.0 x 10^9/L
		Dose interruption until recovery to ≥1.0 x 10^9/L.
		Restart ribociclib at the same dose level.
		• If toxicity recurs at grade 3: temporary dose interruption until recovery to ≥1.0 x 10^9/L.
		  • If resolved in ≤7 days, then maintain dose level.
		  • If resolved in >7 days, then reduce ribociclib dose to the next lower dose level.

	4 <0.5 x 10^9/L	Dose interruption until recovery to ≥1.0 x 10^9/L. Restart ribociclib at the next lower dose level. • If toxicity recurs at grade 4: temporary dose interruption until recovery to ≥1.0 x 10^9/L and reduce ribociclib at the next lower dose level.
Toxicity	**Grade**	**Dose Adjustment and Management Recommendations**
Febrile neutropenia	3 ANC <1.0 x 10^9/L with a single temperature of >38.3°C (101°F) or a sustained temperature ≥38°C (100.4°F) for more than one hour	Dose interruption until improvement of ANC ≥ 1.0 x 10^9/L and no fever. Restart at the next lower dose level. • If febrile neutropenia recurs, discontinue ribociclib.
	4 Life-threatening consequences; urgent intervention indicated	Discontinue ribociclib.
Anemia (Hemoglobin)	1 ≥ 10.0 g/dL	No dose adjustment required.

Toxicity	Grade	Dose Adjustment and Management Recommendations
	2 ≥8.0 – <10.0 g/dL	No dose adjustment required.
	3 <8.0 g/dL	Dose interruption until recovery to grade ≤ 2. Re-initiate ribocliclib at the same dose.
	4 Life-threatening consequences; urgent intervention indicated	Discontinue ribociclib.

6.1.3 Hepatic toxicities

Table 6-6: Recommendations for ribociclib dose modification in case of hepatic toxicities possibly related to study drug

Toxicity	Grade	Dose Adjustment and Management Recommendations
Total bilirubin without ALT/AST increases above baseline value	Grade 1 (> ULN – 1.5 x ULN) (confirmed 48-72h later)	Maintain dose level with LFTs monitored bi-weekly
	Grade 2 (> 1.5 – 3.0 x ULN)	Dose interruption of ribociclib If resolved to ≤ grade 1 in ≤ 21 days, then maintain dose level. Repeat LFTs and bilibrubin tests twice weekly for 2 weeks after resuming treatment. If resolved to ≤ grade 1 in > 21 days or toxicity recurs, then reduce 1 dose level. Repeat LFTs and bilibrubin tests twice weekly for 2 weeks after resuming treatment. • If toxicity recurs after two dose reductions, discontinue ribociclib
	Grade 3 (> 3.0 – 10.0 x ULN)	Dose interruption of ribociclib If resolved to ≤ grade 1 in ≤ 21 days, lower 1 dose level of ribociclib. Repeat LFTs and bilibrubin tests twice weekly for 2 weeks after resuming treatment.If resolved to ≤ grade 1 in > 21 days or toxicity recurs, discontinue ribociclib.
	Grade 4 (> 10.0 x ULN)	Discontinue ribociclib
Confounding factors and/or alternative causes for increase of total bilirubin should be excluded before dose interruption/reduction. They include but are not limited to: evidence of obstruction, such as elevated ALP and GGT typical of gall bladder or bile duct disease, hyperbilirubinemia due to the indirect component only (i.e. direct bilirubin component ≤ 1 x ULN) due to hemolysis or Gilbert Syndrome, pharmacologic treatment, viral hepatitis, alcoholic or autoimmune hepatitis, other hepatotoxic drugs. For patients with Gilbert Syndrome, these dose modifications apply to changes in direct bilirubin only. Bilirubin will be fractionated if elevated.		

Toxicity	Grade	Dose Adjustment and Management Recommendations
AST or ALT without bilirubin elevation > 2xULN	Same grade as baseline or increase from baseline grade 0 to 1 (confirmed 48-72 hrs later)	No dose adjustment required with LFTs monitored per protocol if same grade as baseline or bi-weekly in case of increase from baseline grade 0 to 1
	Increase from baseline grade 0 or 1 to grade 2 (> 3.0– 5.0 x ULN)	Dose interruption of ribociclib If resolved to ≤ baseline value in ≤ 21 days, then maintain dose level. Repeat LFTs and bilibrubin tests twice weekly for 2 weeks after resuming treatment. If resolved to ≤ baseline value in > 21 days or toxicity recurs, then reduce 1 dose level. Repeat LFTs and bilibrubin tests twice weekly for 2 weeks after resuming treatment. • If toxicity recurs after two dose reductions or recovery to ≤ baseline value is > 28 days, discontinue ribociclib
	Increase from baseline grade 0 or 1 to grade 3 (> 5.0– 20.0 x ULN)	Dose interruption of ribociclib until resolved to ≤ baseline value, then lower 1 dose level of ribociclib If recovery to ≤ baseline value is > 28 days, discontinue Ribociclib Repeat liver enzymes and bilirubin tests twice weekly for 2 weeks after dose resumption • If toxicity recurs, discontinue ribociclib
	Increase from baseline grade 2 to grade 3 (>5.0 –	Dose interruption of ribociclib until resolved to < baseline value, then lower 1 dose level of ribociclib. Repeat LFTs and bilibrubin tests twice weekly for 2 weeks after resuming treatment. • If toxicity reoccurs after 2 dose reductions or recovery to < baseline value is > 28 days, discontinue ribociclib
	Grade 4 (> 20.0 x ULN)	Discontinue ribociclib

Toxicity	Grade	Dose Adjustment and Management Recommendations
AST or ALT elevation and concurrent bilirubin elevation	For patients with **normal** ALT or AST or total bilirubin at baseline: • AST or ALT ≥ grade 2 AND • total bilirubin > 2 x ULN without evidence of cholestasis	Discontinue ribociclib
	For patient with **elevated** AST or ALT or total bilirubin at baseline: • [AST or ALT >2 x baseline AND [AST or ALT >3.0x ULN] OR • ALT 8.0x ULN	Discontinue ribociclib
Confounding factors and/or alternative causes for increased transaminases should be excluded before dose interruption/reduction. They include but are not limited to: concomitant medications, herbal preparations or dietary supplements, infection, hepato-biliary disorder or obstruction, new or progressive liver metastasis, and alcohol intake.		

Additional follow-up for hepatic toxicities

Increase in transaminases combined with total bilirubin (TBIL) increase may be indicative of drug-induced liver injury (DILI), and should be considered as clinically important events.

The threshold for potential DILI may depend on the patient's baseline AST/ALT and TBIL value; patients meeting any of the following criteria will require further follow-up as outlined below:

- For patients with normal ALT or AST or TBIL value at baseline: AST or ALT > 3.0 x ULN combined with TBIL > 2.0 x ULN
- For patients with elevated AST or ALT or TBIL value at baseline: [AST or ALT > 2 x baseline AND > 3.0 x ULN] OR [AST or ALT > 8.0 x ULN], whichever is lower, combined with [TBIL > 2 x baseline AND > 2.0 x ULN]

Medical review needs to ensure that liver test elevations are not caused by cholestasis, defined as: ALP elevation > 2.0 x ULN with R value < 2 in patients without bone metastasis, or elevation of ALP liver fraction in patients with bone metastasis.

Note: (The R value is calculated by dividing the ALT by the ALP, using multiples of the ULN for both values. It denotes the relative pattern of ALT and/or ALP elevation is due to cholestatic or hepatocellular liver injury or mixed type injury)

In the absence of cholestasis, these patients should be immediately discontinued from study drug treatment, and repeat LFT testing as soon as possible, preferably within 48 hours from the awareness of the abnormal results. The evaluation should include laboratory tests, detailed history, physical assessment and the possibility of liver metastasis or new liver lesions, obstructions/compressions, etc.

Hepatic toxicity monitoring includes the following LFTs: albumin, ALT, AST, total bilirubin, direct and indirect bilirubin, alkaline phosphatase (fractionated if alkaline phosphatase is grade 2 or higher) creatine kinase, protrombine time (PT/INR) and GGT.

For patients with Gilbert Syndrome: total and direct bilirubin must be monitored, intensified monitoring applies to changes in direct bilirubin only.

Close observation is recommended in case of AST, ALT, and/or bilirubin increase requiring dose interruption, which involves:

- Repeating liver enzyme and serum bilirubin tests **two or three times weekly**. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or return to normal values.
- Obtaining a more detailed history of current symptoms.
- Obtaining a more detailed history of prior and/or concurrent diseases including history of any pre-existing liver conditions or risk factors.
- Obtaining a history of concomitant drug use (including non-prescription medications, herbal and dietary supplements), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis types A, B, C, D, and E; hepatotropic virus infections (CMV, EBV or HSV); autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations.
- Assessing cardiovascular dysfunction or impaired liver oxygenation, including hypotension or right heart failure as possible etiologies for liver dysfunction.
- Liver biopsy as clinically indicated to assess pathological change and degree of potential liver injury.

All cases confirmed on repeat testing meeting the laboratory criteria defined above, with no other alternative cause for LFT abnormalities identified, should be considered as "medically significant", thus met the definition of SAE (Section 8.2.1), and reported as SAE using the term "potential drug-induced liver injury". All events should be followed up with the outcome clearly documented.

6.1.4 Cardiac toxicities

Table 6-7: Dose modification guidance in case of QT prolongation

Toxicity	Grade	Dose Adjustment and Management Recommendations
QTc prolongation	For all grades • Check the quality of the ECG and the QT value and repeat if needed • Perform analysis of serum electrolytes (K+, Ca++, Phos, Mg++). If below the lower limit of normal, correct with supplements or appropriate therapy as soon as possible, and repeat electrolytes until documented as normal. • Review concomitant medication usage for the potential to inhibit CYP3A4 and/or to prolong the QT interval. • Check compliance with correct dose and administration of ribociclib.	
	QTc 450-480 ms	No dose adjustment required.
	QTc 481-500 ms	Interrupt ribociclib Perform a repeat ECG one hour after the first QTcF of ≥ 481 ms If QTcF < 481 ms, restart ribociclib at the same dose. If QTcF remains ≥ 481 ms, repeat ECG as clinically indicated until the QTcF returns to < 481 ms. Restart ribociclib at the same dose level. • If QTcF ≥ 481 ms recurs, ribociclib should be reduced by 1 dose level

	QTc ≥ 501 ms on at least two separate ECGs	Interrupt ribociclib Consider consulting a local cardiologist Perform a repeat ECG one hour after the first QTcF of ≥ 501 ms. If QTcF remains ≥ 501 ms, repeat ECG as clinically indicated, but at least once a day until the QTcF returns to < 481 ms. If QTcF returns to < 481ms, ribociclib should be reduced by 1 dose level. Repeat ECGs 7 days and 14 days after dose resumption for any patient who has therapy interrupted due to QTcF ≥ 501 ms • If QTcF of ≥ 501 ms recurs, discontinue ribociclib
	QT/QTc ≥ 501 **or** > 60 ms change from baseline **and** Torsades de pointes **or** polymorphic ventricular tachycardia **or** signs/symptoms of serious arrhythmia	Interrupt ribociclib Consult a local cardiologist (or qualified specialist) and repeat cardiac monitoring as indicated until the QtcF returns to <481ms • If QTcF returns to < 481ms, ribociclib should be reduced by 1 dose level. Repeat ECGs 7 days and 14 days after dose resumption for any patient who has therapy interrupted due to QTcF ≥ 501 ms. • If QTcF of ≥ 501 ms recurs, discontinue ribociclib

6.1.5 Guidance for Management of All Other Adverse Reactions

Consider performing an analysis of serum potassium, calcium, phosphorus, and magnesium for all adverse reactions that are potentially associated with electrolyte imbalance (e.g. diarrhea, nausea/vomiting). If electrolyte values are below the lower limit of normal, interrupt ribociclib administration, correct electrolytes with supplements as soon as possible, and repeat electrolyte testing until documented normalization of the electrolytes.

Table 6-8: Ribociclib dose adjustment and management recommendation for all other adverse reactions possibly related to study drug

Grade	Dose Adjustment and Management Recommendations
1	No dose adjustment recommended. Initiate appropriate medical therapy and monitor.
2	Dose interruption until recovery to grade ≤1. Initiate appropriate medical therapy and monitor. Re-initiate ribociclib at the same dose. • If the same toxicity recurs at grade 2 within next two cycles (42 days), interrupt ribociclib until recovery to grade ≤1. Re-initiate ribociclib at the next lower dose level.
3	Dose interruption until recovery to grade ≤1. Initiate appropriate medical therapy and monitor. Re-initiate ribociclib at the next lower dose level. • If toxicity recurs at grade 3, discontinue ribociclib.
4	Discontinue ribociclib and treat with appropriate medical therapy.

6.1.6 Interstitial Lung Disease/Pneumonitis

Interstitial Lung Disease (ILD) or pneumonitis is seen as a class effect of CDK4/6 inhibitors. However, several preclinical biochemical and cell-based kinase profiling studies demonstrated that ribociclib is the most selective agent compared to other CDK4/6 inhibitors and animal studies performed with ribociclib in the preclinical setting did not show a signal for pulmonary toxicity.
In the pooled studies (CLEE011E2301, CLEE011F2301, CLEE011A2301), ILD was reported in 0.3% patients and pneumonitis was reported in 0.4% patients in the ribociclib arm (N=1065). In the placebo arm (N= 818), ILD was not reported, and pneumonitis was reported in 0.4% patients. None of ILD or pneumonitis events was serious or led to death. A clinical review of these cases revealed various confounders and no definitive causal relationship with ribociclib could be established.
Additional post-marketing cases of ILD/pneumonitis have been reported including fatalities. Based on the review of the cases, a causal relationship between ribociclib and ILD/pneumonitis has not been established due to multiple confounders and/or limited information.
To minimize the risk of ILD/pneumonitis, monitor patients for pulmonary symptoms indicative of ILD/pneumonitis, which may include hypoxia, cough, and dyspnea. In patients who develop Grade 1 ILD/Pneumonitis, no dose adjustment is required. Initiate appropriate medical therapy and monitor as clinically indicated. In patients who developed ILD/Pneumonitis Grade 2, Kisqali dose should be interrupted until recovery to Grade ≤1, and then Kisqali can be resumed at the next lower dose level. For Grade 3 or 4 permanently discontinue Kisqali.

6.1.7 Toxic Epidermal Necrolysis

In a non-clinical 4-week study performed in dogs, epidermal atrophy of the skin was observed. The atrophy of the epidermis is most likely related to an inhibitory effect of ribociclib on the proliferation of keratinocytes in the basal layer of skin (Miliani de Marval et al 2001). This change was fully reversible after a 4-week treatment-free period.
In the pooled dataset of patients treated with ribociclib plus ET (NSAI or fulvestrant) in Studies CLEE011A2301, CLEE011E2301, and CLEE011F2301 (n=1065), 21.3% of patients reported rash (rash, rash maculopapular, rash pruritic), including 0.9% grade 3/4, compared with 8.6% (no events ≥ grade 3) patients treated with placebo plus ET (n=818). Severe cutaneous reactions such as Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) have not been seen in the clinical development program of ribociclib.
TEN has been reported with Kisqali treatment in post-marketing setting. If signs and symptoms suggestive of severe cutaneous reactions (e.g., progressive widespread skin rash often with blisters or mucosal lesions) appear, Kisqali should be immediately and permanently discontinued.

6.1.8 Adjustment of Starting Dose in Special Populations

Renal impairment

Insufficient data are available to provide a dosage recommendation for ribociclib in patients with renal impairment.

Patients with baseline renal impairment are excluded from the study (serum creatinine > ULN or creatinine clearance < 50 mL/min). Patients who experience a Cr 2-3xULN should hold ribociclib until it resolves to grade 1. If the Cr resolves to grade 1 within 7 days, then maintain dose. If it resolves in >7 days, then lower the dose by 1 dose level. Patients who experience a serum creatinine of grade 2 or higher during the treatment period should discontinue treatment and should be followed for safety assessments.

Elderly

Physicians should exercise caution in monitoring the effects of ribociclib in the elderly. Insufficient data are available to provide a dosage recommendation.

6.2 T-DM1 (Cohort A)

If a patient requires a dose reduction, T-DM1 dosing will be reduced by one dose level per table 6-7 below. Dose re-escalation is not allowed after a dose reduction unless approved by the principal investigator. Indications for details regarding hold parameters as listed in Table 6-8.

Table 6-9: Dose Reduction Levels for T-DM1

Dose Level	**Dose**
Starting Dose	3.6 mg/kg
First reduction	3.0 mg/kg
Second reduction	2.4 mg/kg
Third reduction	Off study

Table 6-10: T-DM1 dose adjustment and management recommendations

Toxicity	**Grade**	**Dose Adjustment and Management Recommendations**
Thrombocytopenia	Grade 1 ($\geq$75 x 10^9/L)	No dose adjustment required.
	Grade 2 ($\geq$50 x 10^9/L - <75 x 10^9/L)	No dose adjustment required.
	Grade 3 ($\geq$25 x 10^9/L - <50 x 10^9/L)	Dose interruption until recovery to grade ≤1. Restart T-DM1 at the same dose level.
	Grade 4 (<25 x 10^9/L)	Dose interruption until recovery to grade ≤1. Restart T-DM1 at the next lower dose level.
Toxicity	**Grade**	**Dose Adjustment and Management Recommendations**

TOTAL BILIRUBIN without ALT/AST increase above baseline value	Grade 1 (> ULN – 1.5 x ULN)	Maintain dose level with LFTs monitored bi-weekly
	Grade 2 (> 1.5 – 3.0 x ULN)	Dose interruption of T-DM1. Once recovers to ≤ grade 1 then restart at same dose level.
	Grade 3 (> 3.0 – 10.0 x ULN)	Dose interruption of T-DM1 Once recovers to ≤ grade 1 then restart at next lower dose level.
	Grade 4 (> 10.0 x ULN)	Discontinue T-DM1
	Confounding factors and/or alternative causes for increase of total bilirubin should be excluded before dose interruption/reduction. They include but are not limited to: evidence of obstruction, such as elevated ALP and GGT typical of gall bladder or bile duct disease, hyperbilirubinemia due to the indirect component only (i.e. direct bilirubin component ≤ 1 x ULN) due to hemolysis or Gilbert Syndrome, pharmacologic treatment, viral hepatitis, alcoholic or autoimmune hepatitis, other hepatotoxic drugs.For patients with Gilbert Syndrome, these dose modifications apply to changes in direct bilirubin only. Bilirubin will be fractionated if elevated.	
AST or ALT without bilirubin elevation > 2 x ULN	Grade 1 (up to 3.0 x ULN)	No dose adjustment required with LFTs monitored per protocol.
	Grade 2 (> 3.0 – 5.0 x ULN)	No dose adjustment required with LFTs monitored per protocol.
	Grade 3 (>5.0 - 20 x ULN)	Dose interruption of T-DM1 until AST/ALT recover to ≤ grade 2, and then restart at next lower dose level.
	Grade 4 (> 20.0 x ULN)	Discontinue T-DM1
AST or ALT AND concurrent Bilirubin elevation > 2 x ULN	AST or ALT ≥ grade 2 (> 5 x ULN) in patients with normal values at baseline and total bilirubin > 2 x ULN OR AST or ALT ≥ grade 3 (> 5 x ULN) in patients with grade 1 or 2 at baseline, and total bilirubin > 2 x ULN	Discontinue T-DM1
	Confounding factors and/or alternative causes for increased transaminases should be excluded before dose interruption/reduction. They include but are not limited to: concomitant medications, herbal preparations or dietary supplements, infection, hepato-biliary disorder or obstruction, new or progressive liver metastasis, and alcohol intake.	

Toxicity	Grade	Dose Adjustment and Management Recommendations
Infusion and Hypersensitivity Reactions	Grade 1-2 Infusion reaction	Reduce infusion rate by 50% or hold till infusion symptoms resolve. Once symptoms resolve, resume at <50% of prior rate. If case of hypersensitivity reaction (severity of infusion increased with subsequent infusion), discontinue T-DM1.
	Grade 3/4 severe infusion/allergic or hypersensitivity reaction	Stop T-DM1. Discontinue T-DM1.
	Infusion-related reactions, characterized by one or more of the following symptoms − flushing, chills, pyrexia, dyspnea, hypotension, wheezing, bronchospasm, and tachycardia. For patients with infusion reactions and/or hypersensitivity reaction, supportive care with medications including (but not limited to) antipyretics, antihistamines, H2 blockers, corticosteroids, or epinephrine, may be used as appropriate at the investigator's discretion. Premedication may be used before subsequent infusions at the investigator's discretion,	
Toxicity	**Grade**	**Dose Adjustment and Management Recommendations**
Pulmonary Toxicity (pneumonitis/interstitial lung disease)	Any Grade	Discontinue T-DM1.
Toxicity	**Grade**	**Dose Adjustment and Management Recommendations**
Cardiac Toxicity Left Ventricular Ejection Fraction (LVEF)	LVEF > 45%	Continue T-DM1.
	LVEF 40 - 45%, and decrease in <10% points from baseline	Continue T-DM1. Repeat LVEF assessment within 3 weeks.
	LVEF 40 - 45%, and decrease in ≥ 10% points from baseline	Hold T-DM1. Repeat LVEF assessment within 3 weeks. If the LVEF is not recovered within 10% points from baseline, discontinue T-

		DM1.
	LVEF < 40%, or symptomatic congestive heart failure	Discontinue T-DM1.
	All patients should have LVEF assessment by ECHO or MUGA atleast every 3 months. This could be obtained earlier as clinically indicated at discretion of treating physician.	
Toxicity	**Grade**	**Dose Adjustment and Management Recommendations**
Peripheral Neuropathy	Grade 1 or 2	Continue T-DM1.
	Grade 3 or 4	Hold T-DM1 till resolves to ≤ grade2, and then restart at next lower dose level.
	Referral to a neurologist should be considered if worsening peripheral neuropathy. Medications for treatment are allowed, unless prohibited in the protocol (Appendix B, table-1).	

6.3 Trastuzumab (Cohorts B and C)

There are no dose modifications for trastuzumab. See Table 6-6 for details regarding hold parameters for cardiac toxicity. Participants who develop grade 4 trastuzumab-related infusion reactions should have trastuzumab permanently discontinued. For delayed or missed doses of trastuzumab on Arm B, if the time between 2 sequential infusions is 28 days or more, the initial dose of 8mg/Kg over 60 minutes should be re-administered as a 60 minute IV infusion followed every 3 weeks thereafter by a dose 6mg/Kg administered over 30-60 minutes. For delayed or missed doses of trastuzumab on Arm C, if the time between 2 sequential infusions is 28 days or more, the initial dose of 6mg/Kg over 60 minutes should be re-administered as a 60 minute infusion followed every 2 weeks thereafter by a dose of 4mg/Kg administered over 30-60 minutes.

6.4 Fulvestrant (Cohort C)

For a toxicity possibly related to fulvestrant, the dose adjustment should be determined by the investigator in accordance with the label. In the event that fulvestrant is discontinued, patients may continue to receive ribociclib and trastuzumab.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1.1 Adverse Events Lists

Please reference Sections 5 and 6 for specific management guidelines for expected toxicities.

7.1.1.1 Adverse Event Lists for ribociclib

Frequent - More than a 10% chance that this will happen

- Leukopenia

- Anemia
- Nausea and/or vomiting
- Diarrhea
- Fatigue
- Stomatitis
- Abdominal pain
- Edema
- Fever
- Constipation
- Back pain
- Headache
- Dyspnea
- Tusis
- Alopecia
- Dermatitis
- Hepatotoxicity
- Vertigo
- Pneumonia

Occasional - Between a 1-10% chance that this will happen

- Thrombocytopenia
- Febrile neutropenia
- Lymphophenia
- Lacrimation increase
- Dry eye
- Dysgeusia.
- Dry mouth
- Oropharyngeal pain
- Dyspepsia
- Increase in blood level creatinine
- Arrhythmia
- Hypocalcemia
- Hypokalemia

- Hypophosphatemia
- Erythema
- Xerosis
- Vitiligo
- Upper abdominal pain
- Stomach ache
- Fainting
- Decreased appetite
- Hepatic failure
- Itchy skin
- Fever
- Urosepsis

Rare - Less than a 1% chance that this will happen

- Respiratory tract infections
- Gastroenteritis
- Sepsis

7.1.1.2 Adverse Event Lists for trastuzumab

Refer to the Investigator's Brochure for detailed trastuzumab information and FDA approved package for more information.
http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/103792s5250lbl.pdf

AE of special interest of trastuzumab are:

- Left ventricular dysfunction
- Infusion associated reactions, hypersensitivity reactions/anaphylaxis
- Embryo fetal toxicity or birth defects
- Respiratory events
- Hematologic events

7.1.1.3 Adverse Events Lists for T-DM1

Refer to the Investigator's Brochure for detailed trastuzumab information and FDA approved package for more information.
http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf

AE of special interest of T-DM1 are:

- Thrombocytopenia
- Liver Function abnormality
- Left ventricular dysfunction

7.1.1.4 Adverse Events Lists for Fulvestrant

Refer to the FDA approved package for specific information on adverse effects of fulvestrant therapy.
https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021344s014lbl.pdf

AE of special interest for fulvestrant are:
- Injection site pain
- Hot flashes
- Musculoskeletal pain

7.2 Adverse Event Characteristics

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

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

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

7.3 Expedited Adverse Event Reporting

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

Serious Adverse Event Definitions

Serious adverse event (SAE) is defined as one of the following:

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect

- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Note that hospitalizations for the following reasons should not be reported as serious adverse events:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (i.e. to perform study related assessments)
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event

Reporting to Novartis

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Novartis within 24 hours of learning of its occurrence.
Any SAEs experienced after this 30 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.
If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study treatment, an oncology Novartis Drug Safety and Epidemiology (DS&E) department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported.

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

Pregnancies

To ensure patient safety, each pregnancy in a patient on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.
Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the oncology Novartis Drug Safety and Epidemiology (DS&E) department. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the Novartis study treatment of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.
Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

7.3.2 DF/HCC Expedited Reporting Guidelines

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

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Expedited Reporting to Hospital Risk Management

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

7.6 Routine Adverse Event Reporting

All Grade 2 or greater Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL AND/OR IMAGING AGENT INFORMATION

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

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

Refer to the appropriate pharmaceutical data sheet, Investigator's Brochure or package insert for agent information.

8.1 Ribociclib

8.1.1 Description

Refer also to Section 2.2.

Include the agent's NSC #
The chemical name is: 7-Cyclopentyl-*N,N*-dimethyl-2-{[5-(piperazin-1-yl)pyridin-2-yl]amino}-7*H*pyrrolo[2,3-*d*]pyrimidine-6-carboxamide succinate (1:1). The molecular formula is C23H30N8O.C4H6O4. The molecular weight is 434.54.

Ribociclib is moderately bound to plasma proteins with the unbound fraction in plasma being approximately 30%. Ribociclib and metabolites are extensively distributed to tissues, but there is no uptake into the brain. Ribociclib is predominantly excreted in the bile as metabolites, with limited excretion of unchanged drug in urine. Mean Cmax (ng/ml) and AUC0-24h (ng*h/ml) for ribociclib are 1090±678(933) and

11300±7220(9720) on Day1, 2290±1420(1940) and 32400±22300(26600) on Day21. (600mg, 3 weeks on 1 week off: LEE011X2107)

Ribociclib is a reversible inhibitor of cytochrome P450 (CYP) enzymes CYP1A2, CYP2E1 and CYP3A4 and a time-dependent inhibitor of CYP3A4. Ribociclib may inhibit these enzymes under therapeutic conditions. No pregnane X-receptor (PXR)-mediated CYP3A4 induction was observed. The *in vitro* inhibitory potency of ribociclib observed for the transporters OATP1B1 (organic anion transporting polypeptide 1B1), BCRP (breast cancer resistance protein), OCT1 (organic cation transporter 1), OCT2, MATE1 (multidrug and toxin extrusion protein 1), MATE2K and BSEP (bile salt export pump) may translate into clinically relevant inhibition at therapeutic doses. Elimination of ribociclib is dominated by oxidative metabolism mainly via CYP3A4 with a minor contribution by flavin-containing monooxygenase 3 (FMO3). The elimination of ribociclib may be affected by co-administered drugs that inhibit or induce CYP3A4. Although ribociclib is a substrate of the P-glycoprotein (P-gp) efflux transporter and subject to active uptake into hepatocytes, these processes are likely not clinically relevant due to the high passive permeability of ribociclib.

8.1.2 Form

Ribociclib is a light tan to yellow powder. Ribociclib will be supplied in the form of 50 mg and 200 mg hard gelatin capsules or film-coated tablets as individually packaged bottles containing 28 capsules or 75 tablets each.

Packaging and labeling

Study Treatment	Packaging	Labeling
Ribociclib (LEE011)	Capsules and/or Tablets in bottles	Labeled as 'LEE011'

8.1.3 Storage and Stability

Study treatments must be received by designated personnel at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated site personnel have access. Upon receipt, the study treatment should be stored according to the instructions specified on the drug labels.

Supply and storage of study treatment

Study Treatment	Supply	Storage
Ribociclib (ribociclib)	Bulk supplied by Novartis	Refer to study treatment label

8.1.4 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 agent.

8.1.5 Availability

Ribociclib will be provided by Novartis Pharmaceuticals at no charge to the study site in the form of 50 mg and 200 mg hard gelatin capsules or film-coated tablets as individually packaged bottles containing 28

capsules or 75 tablets each. Storage conditions are described in the medication label.

8.1.6 Preparation

Participants will be provided with an adequate supply of ribociclib for self-administration at home, including instructions for administration, until at least their next scheduled study visit. Participants will receive ribociclib on an outpatient basis. The investigator shall provide the participant with instructions for ribociclib administration according to the protocol.

Tablets will be dispensed once the capsule supplies are exhausted.

Please dispense a precise count of Ribociclib (LEE011) to patients.

The investigator or responsible site personnel must instruct the participant or caregiver to take ribociclib as per protocol. Ribociclib will be dispensed to the participant by authorized site personnel only. All dosages prescribed to the participant and all dose changes during the study must be recorded on the Dosage Administration Record.

Detailed instructions regarding preparation and administration of ribociclib capsules or tablets will be provided to all Investigator sites separate from this protocol.

8.1.7 Administration

Refer also to Section 5.

Detailed instructions regarding preparation and administration of ribociclib capsules or tablets will be provided to all Investigator sites separate from this protocol.

Compliance should be assessed by the investigator and/or study personnel at each participant visit using information provided by the participant and/or caregiver.

8.1.8 Ordering

Ribociclib will be provided by Novartis Pharmaceuticals.

8.1.9 Accountability

The investigator or designee must maintain an accurate record of the shipment and dispensing of study drug. Drug accountability will be noted by the Novartis field monitor during site visits and/or at the completion of the study. All drug supplies are to be used only for this protocol, and not for any other purpose.

8.1.10 Destruction and Return

Participants must be instructed to return all unused study treatment and packaging on a regular basis, at the end of the study or at the time of study treatment discontinuation.

Returned drug supply by a participant will be destroyed per institutional guidelines following reconciliation by the pharmacy staff.

Any undispensed/unopened investigational drug will be returned to the drug manufacturer or destroyed onsite per institutional guidelines. A member of the research team should notify the research pharmacy at all participating sites at which the trial is being conducted when the last subject on the protocol at that site has completed research drug dosing.

8.2 T-DM1

Refer to the FDA approved package for more information:
http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf

8.2.1 Description

The chemical name is: Tetraamide with N2′ - [3 - [[1 - [(4 - carboxycyclohexyl)methyl] - 2,5 - dioxo - 3 - pyrrolidinyl]thio] - 1 - oxopropyl] - N2′ - deacetylmaytansine, disulfide with human-mouse monoclonal rhuMab HER2 light chain, anti-(human p185neu receptor) (human-mouse monoclonal rhuMab HER2 γ1-chain), immunoglobulin G1 dimer. The molecular formula is $C_{6448}H_{9948}N_{1720}O_{2012}S_{44}•(C_{47}H_{62}ClN_4O_{13}S)_n$.

8.2.2 Form

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

8.2.3 Storage and Stability

T-DM1 should be stored according to the package insert.

8.2.4 Compatibility

The lyophilized product should be reconstituted using Sterile Water for Injection (SWFI). Using a new syringe, 8 mL SWFI should be added to the vial and the vial swirled gently until the product is completely dissolved. The vial should not be shaken. The resulting product contains 20 mg/mL T-DM1, 10 mM sodium succinate, pH 5.0, 60 mg/mL sucrose, and 0.02% (w/v) polysorbate 20. Each 20 mL vial contains enough T-DM1 to allow delivery of 160 mg T-DM1. The reconstituted product contains no preservative and is intended for single use only. The vial should be inspected to ensure the reconstituted product is a clear colorless solution, and is free of particulates before proceeding. Drug from any vial that appears abnormal upon inspection should not be administered to patients. Using a new syringe, the indicated volume of T-DM1 solution should be removed from the vial(s) and added to the IV bag containing at least 250 mL of 0.45% sodium chloride (preferred) or 0.9% sodium chloride injection and gently inverted to mix the solution. A 0.22 micron non-protein adsorptive polyethersulfone (PES) in-line filter is recommended when using 0.45% sodium chloride and required when using 0.9% sodium chloride injection. The solution of T-DM1 should not be shaken. The solution of T-DM1 for infusion should be used immediately. If not used immediately, storage times should not be longer than 24 hours at 2°C−8°C (36°F−46°F) for solutions of T-DM1 diluted in polyvinyl chloride (PVC) or latex-free PVC-free polyolefin, polypropylene, or polyethylene bags containing 0.45% or 0.9% Sodium Chloride Injection, USP.

8.2.5 Handling

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

8.2.6 Availability

T-DM1 is commercially available as KADCYLA™.

8.2.7 Preparation and Administration

T-DM1 should be prepared and administered per instructions in the KADCYLA™ package insert. T-DM1 will be administered intravenously under the direction of the Investigator.

8.2.8 Ordering

T-DM1 will be obtained through commercial supply. Check with the site Director of Pharmacy and/or the site research pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered **before** the protocol is activated.

8.2.9 Accountability

The investigator or designee will comply with all institutional commercial drug SOPs.

8.2.10 Destruction and Return

Any remaining T-DM1 and used vials will be destroyed on site after preparation per the Institutional Pharmacy SOP.

8.3 Trastuzumab

Refer to the FDA approved package for more information:
http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/103792s5250lbl.pdf

8.3.1 Description

Refer also to Section 2.3.

The molecular formula is C6470-H10012-N1726-O2013-S42. The molecular weight is 145532.

Trastuzumab pharmacokinetics are dose-dependent. Mean half-life increases and Cl decreases with increasing dose level. Mean half-life is 16 days (range, 11 to 23 days) after an initial dose of 8 mg/kg followed by a dose of 6 mg/kg every 3 weeks. Between weeks 6 and 37, trastuzumab serum concentrations

reached a steady-state with mean trough and peak concentrations of 63 mcg/mL and 216 mcg/mL, respectively.

8.3.2 Form

Trastuzumab is a sterile, white to pale yellow, preservative free lyophilized powder for intravenous (IV) administration. Vials may be supplied by Genentech in either 440mg or 150mg dosages (may vary upon commercial availability). Each 440mg vial of trastuzumab contains 440 mg of trastuzumab, 9.9 mg of l-histidine HCl, 6.4 mg of L histadine, 400 mg of α, α trehalose dihydrate, and 1.8 mg of polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI) USP, containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6. The reconstituted formulation (440mg vial) is designed for multiple uses. Unused drug may be stored for 28 days at 2ºC-8ºC (36ºF-46ºF).

8.3.3 Storage and Stability

Vials of trastuzumab are stable at 2°C-8°C (36°F-46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of trastuzumab reconstituted with BWFI is stable for 28 days after reconstitution when stored refrigerated at 2°C-8°C (36°F-46°F), and the solution is preserved for multiple use. Discard any remaining multi dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. **DO NOT FREEZE TRASTUZUMAB THAT HAS BEEN RECONSTITUTED.**

The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% sodium chloride for injection, USP, may be stored at 2°C-8°C (36°F-46°F) for up to 24 hours prior to use. Diluted trastuzumab has been shown to be stable for up to 24 hours at room temperature 15°C-25°C; however, since diluted trastuzumab contains no effective preservative the reconstituted and diluted solution should be stored refrigerated (2°C-8°C).

8.3.4 Compatibility

No incompatibilities between trastuzumab and polyvinylchloride or polyethylene bags have been observed. Trastuzumab should not be mixed or diluted with other drugs. Trastuzumab infusions should not be administered or mixed with Dextrose solutions. Trastuzumab should not be filtered during administration.

8.3.5 Handling

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

Trastuzumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through drug accountability documentation, as well as the recording of

study treatment administration in the subject's medical record. In addition, all details pertaining to the administration of trastuzumab, including but not limited to date, actual dose, and start and end times of each dose, will be recorded on the Study Drug Administration page of the subject's eCRF.

CRA's will review treatment compliance during investigational site visits conducted during and at the completion of the study.

8.3.6 **Availability**

Trastuzumab is commercially available. This will not be free of charge but will be given as part of routine clinical care.

8.3.7 **Preparation**

Use appropriate aseptic technique. Each 440mg vial of trastuzumab should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multidose solution containing 21 mg/mL trastuzumab. Immediately upon reconstitution with BWFI, the vial of trastuzumab must be labeled in the area marked "Do not use after" with the future date that is 28 days from the date of reconstitution.

Each 150 mg vial is reconstituted with 7.4 mL of Sterile Water for Injection (SWFI). The reconstituted solution contains 21 mg/mL trastuzumab and will be added to 250 ml of 0.9% Sodium Chloride Injection, USP. Use the Herceptin solution immediately following reconstitution with SWFI, as it contains no preservative. If not used immediately, store the reconstituted Herceptin solution for up to 24 hours at 2ºC-8ºC; discard any unused Herceptin after 24 hours.

If the patient has known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with sterile water for injection. Trastuzumab that has been reconstituted with SWFI must be used immediately and any unused portion discarded. Use of other reconstitution diluents should be avoided. Determine the dose of trastuzumab needed, based on a loading dose of 8 mg trastuzumab/kg body weight for q3wk dosing schedules or a maintenance dose of 6 mg/kg trastuzumab/kg body weight for q3w dosing schedules. Calculate the correct dose using 21 mg/mL trastuzumab solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

8.3.8 **Administration**

Refer to Section 5

8.3.9 **Ordering**

Trastuzumab is a commercially available. Check with the site Director of Pharmacy and/or the site research pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered **before** the protocol is activated.

8.3.10 Accountability

The investigator or designee will comply with all institutional commercial drug SOPs.

8.3.11 Destruction and Return

Any remaining Trastuzumab and used vials will be destroyed on site after preparation per Institutional Pharmacy SOP.

8.4 Fulvestrant

8.4.1 Description

Fulvestrant injection for intramuscular administration is an estrogen receptor antagonist. The chemical name is 7-alpha-[9-(4,4,5,5,5-pentafluoropentylsulphinyl) nonyl] estra-1,3,5-(10)- triene-3,17- beta-diol. The molecular formula is $C_{32}H_{47}F_5O_3S$.

8.4.2 Form

A 500mg dose is supplied as two 5mL clear neutral glass (Type 1) barrels, each containing 250mg.5mL of fulvestrant solution for IM injection and fitted with tamper evident closure.

8.4.3 Storage and Stability

Fulvestrant syringes are presented in a tray with polystyrene plunger rod and safety needles for connection to the barrel. Store at between 2-8 degrees Celsius. To protect from light store in the original carton until time of use.

8.4.4 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 fulvestrant in a self-contained and protective environment.

8.4.5 Availability

Fulvestrant is available commercially as FASLODEX™.

8.4.6 Preparation and Administration

Fulvestrant should be prepared and administered per instructions in the FASLODEX™ package insert. T-DM1 will be administered intramuscularly under the direction of the Investigator.

8.4.7 Ordering

Fulvestrant will be obtained through commercial supply. Check with the site Director of Pharmacy and/or

the site research pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered **before** the protocol is activated.

8.4.8 Accountability

The investigator or designee will comply with all institutional commercial drug SOPs.

8.4.9 Destruction and Return

Any remaining Fulvestrant and used syringes will be destroyed on site after preparation per the Institutional Pharmacy SOP.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

Cohort A
Biomarker assessments will be performed to identify molecular signatures that may be associated with response or resistance to treatment. Tissue samples may be used to assess HER2 or other relevant protein levels or genetic changes to tumor DNA that may confer resistance or sensitivity to ribociclib therapy.

Potential predictive biomarkers
Patients must supply a tumor specimen that may be from a previous biopsy (archival tumor specimen) or from a newly obtained tumor specimen. The most recent biopsy is preferred. The availability of tumor specimens must be confirmed before the patients receive treatment. Additionally, a pathology report must be submitted along with the patient's archival tumor block/slides.

Baseline tumor samples (archival or newly obtained) will be tested retrospectively for Rb protein and PTEN protein by immunohistochemistry. The status of molecules (e.g., gene expression, mutations, amplifications, deletions and/or protein expression/activation etc.) that are involved in the D-cyclin-CDK4/6-INK4a-Rb and mTOR pathways, such as mutations of CCNDl , PIK3CA, PTEN and CDK4; gene amplification of CCNDl and CDK4, deletion of CDKN2, as well as other cancer associated genes, will also be investigated in the tumor tissue from all patients (provided that acceptable assays exist), with the intention to identify potential predictive markers related to therapeutic responses. The mutations will be analyzed utilizing the institutional tumor genotyping next generation sequencing (NGS) assay known as “Snapshot-NGS assay” on DNA isolated from the tumor. This assay was developed within the CLIA certified MGH pathology laboratory and utilizes a multiplex polymerase chain reaction (PCR) technology called Anchored Multiplex PCR (AMP) for single nucleotide variant (SNV) and insertion/deletion (indel) detection in genomic DNA[40]. Briefly, for detection of mutations by the Snapshot-NGS assay, the genomic DNA is sheared with the Covaris M220 instrument, followed by end-repair, adenylation, and ligation with an adapter. A sequencing library targeting hotspots and exons in 39 genes is generated using two hemi-nested PCR reactions. Illumina MiSeq 2 x 151 base paired-end sequencing results are aligned to the hg19 human genome reference using BWA-MEM. MuTect and a laboratory-developed insertion/deletion analysis algorithm are used for SNV and indel variant detection, respectively. This assay has been validated to detect SNV and indel variants at <5% allelic frequency or higher in target regions with sufficient read coverage. Variants are reported with Human Genome Variation Society (HGVS) protein and DNA nomenclature, followed by the referenced Ensembl transcript ID. The gene targets represent key exons targeted for somatic mutations in human cancer. The assay also includes complete

coding exon coverage for tumor suppressor genes. Other pathways that may interact with Dcyclin-CDK4/6- INK4a-Rb and/or mTOR, or thought to be important in cancer may also be assessed, depending on sample and assay availability. The results from these exploratory analyses will be correlated with clinical outcome to determine potential predictive biomarkers of ribociclib /T-DM1 response.

Pharmacodynamic biomarker assessments
Optional tumor samples (if safely accessible and feasible) will be taken pre- and post-treatment in order to assess dose-dependent target modulation.

The pre-treatment biopsy sample may be taken anytime during the molecular pre-screening or screening period. If a new biopsy is performed during the molecular pre-screening period to assess total pRb status, this sample can serve as the pre-treatment sample in the paired pre- and post-treatment analysis. The post-treatment sample can be taken at any time in Cycle 2 Day 10-18. Although there is flexibility in the post-treatment biopsy collection day, it is critical to obtain the sample within 6 hours of the ribociclib dose (obtaining the tumor tissue biopsy 4-6 hours after the ribociclib dose is preferred, in order to have the best opportunity to measure the ribociclib 's effects on p-pRb and Ki67). The frozen biopsy specimen will be utilized for analysis of gene expression profile of key genes in cyclin-D pathway.

Table 9-1 Cohort A Biomarker sample collection schedule

Sample	Tissue	Visit	Biomarker assay
FFPE Tumor	Tumor	Screening	RB and PTEN by IHC Next generation sequencing including PIK3CA, CDK4, PTEN, CCND1 Copy number assessment of CDK4, CCND1, CDKN2
Newly obtained, optional matched pre- and on-therapy tumor	Tumor	Screening and Cycle 2 day 10-18	Total and pRB, Ki-67, p-Akt, p-S6K and caspase-3 expression by IHC Gene expression profile of key genes in cyclin-D pathway
Newly obtained, optional	Tumor	Screening and post- progression	Next generation sequencing including PIK3CA, CDK4 ,PTEN, CCND1 Copy number assessment of CDK4, CCND1, CDKN2

Optional biomarker assessment at time of disease progression
If a patient enrolls on the optional study of the mechanisms of resistance, there will be additional new tumor samples required at time of disease progression. These would be analyzed for mutations utilizing the institutional tumor genotyping NGS assay (Snapshot-NGS assay) as described above. Copy number assessment of CDK4, CCND1, CDKN2The pre-treatment biopsy may coincide with the baseline tumor biopsy collected for pharmacodynamic testing, as described above.

Other exploratory biomarker assessments
During the study, in addition to the biomarkers specified above, exploratory research may be conducted on any tumor and blood (including PK) samples. These studies would extend the search for other potential biomarkers relevant to the effects of ribociclib and T-DM1 and/or prediction of these effects and/or resistance to the treatment, and/or safety. This may include the development of ways to detect, monitor or treat cancer. These additional investigations would be dependent upon clinical outcome, reagent and sample availability.

While the goal of the biomarkers is to provide supportive data for the clinical study, there may be circumstances when a decision is made to stop a collection, or not perform or discontinue analysis due to either practical or strategic reasons (e.g., inadequate sample number, issues related to the quality of the sample or issues related to the assay that preclude analysis, impossibility to perform correlative analyses, etc.). Therefore, depending on the results obtained during the study, sample collection/analysis may be omitted at the discretion of Novartis.

If the patient agrees, any remaining samples (tumor) may be stored for up to 15 years and further analyzed to address scientific questions related to ribociclib, T-DM1 and/or cancer. This may include the development of ways to detect, monitor or treat cancer. The decision to perform such exploratory biomarker research studies would be based on outcome data from this study or from new scientific findings related to the drug class or disease, as well as reagent and assay availability.

Cohorts B and C
Tumor biopsies will be performed at baseline and cycle 2 day1 (C2D1), and tissue obtained will be both fixed in formalin for paraffin embedding and frozen fresh. CTCs will be drawn at baseline for correlative studies below.

1. Phospho-Rb and total Rb:

Through inhibition of CDK4/6, the primary pharmacodynamic marker of ribociclib activity is a reduction in phosphorylation of the retinoblastoma (Rb) tumor suppressor protein. A reduction in Rb phosphorylation mitigates the activity of the E2F family of transcription factors, the canonical role of which is to facilitate cell cycle progression through the G1/S checkpoint[41]. Given that cyclin D1 lies downstream of HER2, it is also possible that trastuzumab might invoke a slight reduction in Rb phosphorylation as well[42].

Formalin-fixed, paraffin embedded (FFPE) tissue from baseline and C2D1 biopsies will be stained by standard immunohistochemical techniques for both p-Rb (ser780 residue) and total Rb. These techniques are already optimized through the histopathology core facility at the Brigham and Women's Hospital, Boston MA. For p-Rb, sections will be scored manually and the percentage positive cells will be annotated; a minimum of 200 cells will be counted. Additionally, stained slides will be scanned using an Aperio ScanScope XT workstation (Aperio Technology, Inc., Vista, CA). Images will be visualized and digitally annotated (as regions of interest, ROIs) using ImageScope software (version 10.0.35.1800, Aperio Technology). The ROIs will then be analyzed using a standard analysis algorithm to quantitate the proportion of area that is positive for staining (color deconvolution v9.0, Aperio Technology).

We expect to see a reduction in Rb phosphorylation in response to treatment with trastuzumab plus ribociclib. A significant reduction in p-Rb would serve as a pharmacodynamic marker of drug activity. We will also perform exploratory studies to determine if patients deriving clinical benefit from the regimen (CR, PR, or SD) show a greater reduction in Rb phosphorylation in response to trastuzumab plus ribociclib in comparison to patients with primary disease progression.

Based on our laboratory data, we do not expect a significant change in total Rb levels after treatment with trastuzumab plus ribociclib. However, preclinical data suggests that Rb-deficient tumors might exhibit an impaired response to CDK4/6 inhibition, presumably as they grow in an Rb-independent fashion[43]. We will determine if response rates to trastuzumab plus ribociclib differ between Rb-proficient versus Rb-deficient tumors.

2. Beta-galactosidase and cleaved caspase-3

Preclinical data suggests that inhibition of the cyclin D-CDK axis in tumor cells can induce either cellular apoptosis or senescence. In mouse models, the cellular phenotype after CDK4/6 inhibition has varied according to tumor type, with solid tumors including mammary carcinoma demonstrating a senescence response, and hematologic malignancies demonstrating massive apoptosis[18]. Whether these findings are recapitulated in human cancers is unknown. Indeed, clinical evidence of dramatic breast cancer shrinkage after a relatively short period of CDK4/6 inhibition suggests that apoptosis might be a predominant mechanism of cellular death[20].

We will perform immunohistochemical staining for beta-galactosidase (a marker of cellular senescence) and cleaved caspase-3 (a sensitive marker for apoptosis) on FFPE tissue from biopsies obtained at baseline and at C2D1. Quantification and analysis of stained slides will be performed as described above.

We will determine if there is a significant change in beta-galactosidase, cleaved caspase-3, or both when comparing biopsies at baseline and a C2D1. The data might provide insights into differential cellular mechanism(s) of response to combined anti-HER2 therapy and CDK4/6 inhibition.

3. FoxM1

Recently, the Forkhead box protein M1 (FoxM1) transcription factor has been identified as a downstream phosphorylation target of CDK4/6[44]. CDK4/6-induced phosphorylation of FoxM1 maintains expression of G1/S phase genes and protects against cellular senescence, thus promoting a malignant phenotype. Although it has not been previously studied, it is possible that pharmacologic CDK4/6 inhibition within human tumors could reduce p-FoxM1 levels, in part explaining its therapeutic activity.

At present, primary antibodies for reliable immunohistochemistry against phosphorylated FoxM1 are not commercially available. As such, we would derive protein lysates from tumor core biopsy tissue that was frozen at baseline and at C2D1 (only cores with confirmed >50% tumor cells will be used). Western blotting will be performed for p-FoxM1 (ser35) and total FoxM1. We will determine whether combined trastuzumab and ribociclib reduces p-FoxM1 levels, and whether patients exhibiting a response to therapy are more likely to have a reduction in FoxM1 phosphorylation.

4.Genomic analyses

In this trial, all core biopsies with tumor cell cellularity >50% (as determined by frozen section histology) will be submitted for DNA extraction, followed by next-generation, massively parallel, paired end, whole exome sequencing (WES) at the Broad Institute. We will use the data obtained to interrogate specific biological hypotheses as well as perform exploratory analyses.

a. Copy number for CCND1, CCNE1, CDKN2A

Approximately one-third of HER2-positive breast cancers show amplification of the CCND1 gene (encoding cyclin D1), and 5% show amplification of the CCNE1 gene (encoding cyclin E). Theoretically, amplification of CCND1 might lead to an increase in Rb phosphorylation, heightened dependence on the cyclin D1-CDK4 pathway, and increased sensitivity to CDK4/6 inhibition. Conversely, amplification of CCNE1 (which complexes with CDK2 to hyperphosphorylate Rb) is a proven mechanisms of trastuzumab resistance[45] and could serve as a mechanism of resistance to CDK4/6 inhibition as cells have recruited alternate mechanisms to transition through the G1/S checkpoint[46]. Finally, approximately 5% of HER2-positive breast cancers demonstrate homozygous deletion of the CDKN2A gene, which encodes the CDK4 inhibitor p16. These cancers might also harbor heightened dependence on CDK4/6.

Using in-house computational algorithms, we will determine copy number for each of these genes in

the baseline biopsies taken from patients entering on study. We will determine whether the presence of CCND1 amplification or CDKN2A deletion is associated with increased response to trastuzumab plus ribociclib, and similarly whether CCNE1 amplification is associated with treatment-resistance. Given the low frequencies of CDKN2A loss and CCNE1 amplification in HER2-positive tumors, it is most likely that this analysis will only be sufficiently powered to make statistically significant conclusions for the CCND1 analysis.

b. TP53 mutation status
Approximately 50% of HER2-positive breast cancers harbor mutations in TP53. An exploratory analysis of tumor tissue (ER-positive, predominantly HER2-negative) from patients treated with the CDK4/6 inhibitor abemaciclib suggested that TP53 mutations are associated with lack of response[20]. We will determine TP53 mutation status in all tumor biopsies (at baseline) and determine if there is an association in HER2-positive patients between TP53 mutation status and response to trastuzumab plus ribociclib.

c. Exploratory analysis of WES data
In conjunction with our collaborators at the Broad Institute, we will also perform exploratory analyses to describe other exomic mutations that correlate with response or resistance to ribociclib plus trastuzumab. The data obtained will be considered exploratory and might serve to generate hypotheses to be pursued in future studies.

5. Circulating tumor cells (CTCs)

Blood will be drawn at baseline for CTC isolation. The correlation between CTC Rb status and tumor Rb status will be examined. We will also analyze CTC DNA as another approach to determine Cyclin D1, Cyclin E1, and CDKN2A copy number levels. The primary advantage of this technique is that malignant cells are collected via purification of circulating tumor cells (CTCs) from whole blood, which is a far less invasive technique for malignant cell sampling than traditional biopsies. Interestingly, these CTCs are indicative of tumor metastases and predictive of clinical outcomes in breast cancer patients. Patients will be grouped as to whether or not their tumor has amplified CCND1, CCNE1, or loss of CDKN2A and response to therapy evaluated.

CTC collection will be performed from whole blood samples using the Automated CellSearch™ processing technique (Veridex). Briefly, 7.5ml of whole blood is collected in CellSave tubes™. At baseline **2 tubes** of whole blood will be collected in CellSave tubes™ for CTC analysis. Samples must be kept at room temperature and must be shipped/dropped off within same day. Please do not draw samples on Fridays.

9.1.1.1 Collection of CTC Specimen(s)

1. Ensure that peripheral blood collection occurs prior to administration of i.v. therapy.
2. The blood sample must be collected in a CellSave preservative tube. Label the tube with the sample identifier/number, protocol number, and submitting investigator.
3. **2 tubes** of whole blood will be collected. Collect at least 8ml of blood for 1 tube. Gently invert the tube 8 times to prevent clotting immediately after filling the tube.
4. Do not submit clotted samples.

9.1.1.2 Handling and Shipping of CTC Specimen(s)

1. The blood sample must be transported and stored at room temperature (15-30C) until processing. Do NOT refrigerate or freeze the sample.
2. Samples must be shipped/dropped off within the same day.
3. Ship the tube with completed requisition form **AT ROOM TEMPERATURE** to:

Dana-Farber Cancer Institute
Attn: Laura Spetalnick, Krishan Taneja, Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

9.1.1.3 Site(s) Performing CTC Correlative Study

CTC blood samples will be processed by the laboratory of Dr Alarice Lowe at the Brigham and Women's Hospital (Medical Research Building, 3rd floor). The tubes will be delivered to the lab by the Clinical Research Coordinator together with a requisition form.

After CTC isolation, samples will be transferred to the laboratory of Cloud Paweletz for further analysis. This should be done on the same day as processing.

6. Circulating cell free DNA

Blood will be collected at baseline, restaging visits and at time of progression for evaluation of cell-free DNA (cfDNA). The cfDNA will be processed by the Clinical Research Laboratory (CRL) at DFCI and then banked in the DF/HCC Clinical Trials Core laboratory for future research purposes. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.1.1.4 Collection of cfDNA specimen(s)

One 10 ml of whole blood will be collected in Streck Tubes. The blood sample will be collected and processed at baseline, restaging visits and time of progression for evaluation of cfDNA. Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.1.1.5 Handling and shipping of cfDNA specimens

One 10 ml Streck tube will be collected and processed at baseline, restaging visits and at time of progression for evaluation of cfDNA. Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results. Ship within 24 hours of collection at ambient temperature overnight **to**:

Dana-Farber Cancer Institute
Attn: Laura Spetalnick, Krishan Taneja, Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

Tube precautions:

- If samples cannot be shipped within 24 hours of collection, contact DFCI. DO NOT FREEZE OR REFRIDGERATE TUBES as this could result in cfDNA breakage. Blood collected in the Streck tube can be stored for 14 days between 6-37 degrees Celsius.
- 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 analytical results.

Shipping Note: Streck tube samples are sent ambient. Frozen and ambient specimens obtained and shipped on the same day to the DFCI blood bank (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.

See Appendix F for additional instructions on use of Streck tubes.

Table 9-2 Cohorts B and C Biomarker sample collection schedule

Sample	**Type**	**Visit**
Archival FFPE Tissue	Tumor	Screening
Newly obtained biopsy	Tumor	Screening and Cycle 2 day 1
cfDNA	Blood	Screening, Restaging Visits and Progression
CTC	Blood	Screening

9.2 Laboratory Correlative Studies

9.2.1 Pharmacokinetic Measures (Cohort A)

Plasma and serum serum samples to assess the effect of combination treatment on the PK of ribociclib will be collected. Plasma samples will be collected in all patients participating to measure levels of ribociclib and its metabolite. The analysis will be done core-lab under guidance of Dr. Supko. Table 9-1 describes the ribociclib PK blood sample schedule.

Table 9-3 PK Blood Sample Schedule

Cycle	Day	Time point	PK Ribociclib
1	8	0h (pre-dose) of ribociclib	1 x 5 ml
		0.5 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		1 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		2 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		4 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		6 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		8 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
1	9	24 hours (±30 minutes) following dosing of ribociclib on Day 8 (and prior to Cycle 1 Day 9 dosing of ribociclib)	1 x 5 ml
1	21	0h (pre-dose)	1 x 5 ml
		0.5 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		1 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		2 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		4 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		6 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
		8 h (±5 minutes) following dosing of ribociclib	1 x 5 ml
2	1	24 hours (±30 minutes) following dosing of ribociclib on Day21	1 x 5 ml
		0h (pre-dose) of T-DM1	1 x 5 ml

9.2.2 Guidelines for Tissue Acquisition and Biopsies of Metastatic Lesions

9.2.2.1 Collection of Specimen(s)

Cohort A: at baseline, C2D10-18, time of progression (optional)

Cohorts B and C: at baseline and C2D1 (mandated for patients with accessible disease)

Tissue specimens will be collected from recurrent or metastatic lesions using standard institutional procedures. The amount of tissue collected will follow the guidelines listed below. If a participant has more than one site of disease, only one site needs to be biopsied in order to go on to the study and the site is left to the discretion of the patient and their 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 for the baseline tissue sample. Participants who undergo a research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are still eligible and are not required to undergo a repeat biopsy in order to enter the study. Research biopsies can be waived, with PI approval, in participants who do not have easily accessible disease for Cohort B and Cohort C.

Breast: A goal of 3-6 core biopsy specimens will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass.

Skin/chest wall: A goal of 1-2 5-mm punch biopsies will be obtained.

Lymph node: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules will be performed on this protocol, unless they are clinically indicated.

Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a participant has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 11-13gaugeneedle.

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.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue stored for research at the time of the procedure (provided that the tissue is processed as specified in Section 9.2.1.2), in which case, the participant would not be required to undergo a separate research biopsy for entry into this protocol.

9.2.2.2 Handling of Specimens(s)

Core biopsy specimens will be handled and processed at the time of biopsy collection. Ideally, sufficient core biopsy samples will be obtained to allow for some to be frozen (after embedding in OCT) and others to be fixed in formalin and subsequently embedded into paraffin blocks. The specific instructions for handling core biopsy material is provided in Appendix E.

9.2.2.3 Shipping of Specimen(s)

Cohort A
With the help of the clinical research assistant/coordinator (CRA/CRC), the specimens will be shipped to the Translational Research Lab (TRL) at *Massachusetts General Hospital* (address provided in Appendix E) to perform biomarker testing. Patients must supply a tumor specimen that may be from a previous biopsy (archival tumor specimen) or from a newly obtained tumor specimen. The most recent biopsy is preferred. Additionally, a

pathology report must be submitted along with the patient's archival tumor block/slides. The specimens will be labeled with a unique specimen identification number, protocol number, patient study registration number, date of biopsy, and site of biopsy. The data will be stored in a password protected hard-drive.

Cohort B and Cohort C
Specimens will be shipped to the DF/HCC Clinical Trials laboratory at the following address:

Dana-Farber Cancer Institute
Attn: Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

Participants will be informed that their specimens may be used for research by investigators at DF/HCC and other approved collaborators. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed. Correlative studies described in Section 9.1 will be performed by Shom Goel in the Department of Cancer Biology at DFCI or relevant core facilities at DFCI.

Genomic sequencing as described will be performed at the Broad Institute.

9.2.2.4 Site(s) Performing Correlative Study

Cohort A
Massachusetts General Hospital

Cohort B and Cohort C
Dana-Farber Cancer Institute

9.2.2.5 Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:

Breast (core biopsy):

- 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, liver, or bone (core needle biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, damage to adjacent organs. Additional risks may be present if

intravenous 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 separate procedure consent. Patients will be evaluated for comorbidities 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.

9.2.2.6 Risks of Anesthesia

Local Anesthesia

All biopsy procedures require local anesthesia using lidocaine, xylocaine, 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 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[47]. 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 *arenot 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.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. 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.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

Table 10-1 Study calendar for Cohort A

	Screening	**Cycle 1**					**Cycle 2**			**Cycle 3+**		**End of Tx**	**30 Day F/U**
Day of cycle		D1	D8	D 9	D 15	D 21[l]	D1	D8	D15	D1	D21 +/- 7		
Informed consent	X												
Study eligibility	X												
HER2 status	X												
Physical exam	X[a]	X[g]	X		X	X		X	X	X		X	
Vital signs	X	X	X		X	X		X	X	X		X	
ECOG PS	X	X[g]	X		X	X		X	X	X		X	
Labs[b]	X	X[g]	X		X	X		X	X	X		X	
PT-INR	X												
PK sampling			X	X		X	X						
Urinalysis	X	X	X			X		X		X		X	
CT/MRI[c]	X								X[h]		X[h]	X[h]	
ECG[k]	X	X[g]	X		X	X		X	X	X		X	
ECHO/MUGA	X										X[i]	X	
Pregnancy test[d]	X												
Hepatitis B and C Test	X												
Con Meds	X	X	X		X	X		X	X	X		X	
Medical History	X												
AE Assessment		X	X		X	X		X	X	X		X	X
Ribociclib[e]			X----------X					X[e]					
T-DM1[f]		X					X			X			
Archival Tissue	X												
Optional Biopsy	X								X			X[j]	

a. Include breast exam.
b. Blood samples for hematology, clinical chemistry (including calcium, magnesium, and phosphorus), and liver function tests (albumin, ALT, total protein, alkaline phosphotase, AST, and total bilirubin). HBsAg, anti-HBC, and Anti-HCV should be done at screening only. Cycle 1 Day 1 labs do not need to re-meet eligibility criteria.
c. CT/MRI scan of all known sites of disease and assessment of tumor burden as per RECIST1.1. Additional

imaging, such as nuclear bone scans, may also be done as appropriate at the discretion of the Investigator.

d. Pregnancy test within 7 days prior to treatment (not required for males or females of non-child-bearing potential).
e. Ribociclib is administered PO on day 8-21. During cycles with Day 8 and 15 visits, the dose of ribociclib is required to be administered in the clinic.
f. T-DM1 is administered IV, once every 21 days.
g. These evaluations do not need to be repeated if a patient is screened within 96 hours of scheduled first dose of T-DM1, with the exception of liver function tests, which must be repeated on Cycle 1 Day 1 regardless of when taken for screening. Results mast be reviewed and confirmed that patient is still prior to first dose.
h. Tumor Assessments should be done with the same imaging modality as screening and will be performed at baseline, the end of cycles 2, 4, 6, and 8, and then at the end of every 3rd cycle (i.e. cycles 11, 14, 17, etc.). End of treatment only required if not done within the previous 2 cycles (or 3 cycles if beyond C11). Imaging may be performed sooner if there is clinical concern for progression.
i. ECHO or MUGA using the same cardiac testing modality performed at screening will be performed at the end of every 4th cycle (i.e. Cycle 4, 8, 12, etc.).
j. If the patient consents, the optional biopsy should be done at the time of progression.

k. A standard 12-Lead ECG. For patients with QTcF ≥481 ms at any time, interrupt study treatment and follow the procedures described in the "Ribociclib Dose Modification section". If treatment is resumed, repeat ECGs 7 days and 14 days after dose resumption (and then as clinically indicated). During subsequent cycles, perform predose ECG for every cycle starting at cycle 6, and predose and 2-4 postdose starting at cycle 9 and every 3rd cycle thereafter.
l. Assessments completed on Cycle 1 Day 21 do not need to be repeated on Cycle 2 Day 1.

Table 10-2 Study calendar for Cohort B, Phase Ib Study

	Screening[a]	Cycle 1			Cycle 2+		End of treatment
		D1	D8	D15	D1	D20	
Ribociclib[b]		X			X		
Trastuzumab[c]		X			X		
Informed consent	X						
Inclusion/Exclusion Criteria	X						
Demographics	X						
Medical history	X						
ConMeds	X	X	X	X	X		
Physical exam	X	X			X		X
AE assessment	X	X	X	X	X		X
Vital signs	X	X			X		X
Height	X						
Weight	X	X			X		
Performance status	X	X			X		X
Labs[d]	X	X	X	X	X		X
PT-INR	X	X			X		X
Urinalysis/BUN	X	X			X		X
Lipid Panel	X				X[e]		
ECG[k]	X	X	X[f]	X[f]	X[f]		X
MUGA/ECHO	X					X[g]	
Tumor assessments	X					X[h]	X
Pregnancy Test	X						
Hepatitis B and C Test	X						
Research Biopsy	X				X[i]		
Blood for cfDNA[j]	X				X[j]		X
CTC	X						
Survival Follow-up							X

a. Screening Imaging (CT, MRI and ECHO/MUGA) can be done within 28 days of starting protocol therapy. Screening labs should be done within 14 days of starting protocol therapy.
b. Ribociclib is given continuously Days 1-21 of a 21 day cycle except for at Dose Level -2 where Ribociclib is given Day 1-14 of a 21 day cycle.

c. Trastuzumab should be administered within +/- 3 days of the scheduled assessment unless otherwise noted.
d. Labs to include: hematology, clinical chemistry (including calcium, magnesium, and phosphorus), and liver function tests (albumin, ALT, total protein, alkaline phosphotase, AST, and total bilirubin). Cycle 1 Day 1 labs do not need to re-meet eligibility criteria.
e. Lipid Panel to be drawn on Day 1 every 4 cycles beginning at screening (i.e. screening, then day 1 of cycles 5, 9, 13, etc).
f. ECGs on Cycle 1 Days 8 and 15 should be done pre-dose, and 2-4hours post dose. Beginning with Cycle 2, ECGs should be done every 2 cycles (i.e. cycles 2, 4, 6, etc.).
g. ECHO or MUGA using the same cardiac testing modality performed at screening will be performed at the end of every 4th cycle (i.e. Cycle 4, 8, 12, etc.).
h. Tumor Assessments should be done with the same imaging modality as screening and will be performed at screening, the end of cycles 2, 4, 6, and 8 and then at the end of every 3rd cycle (i.e. cycles 11, 14, 17, etc.). End of treatment only required if not done within the previous 2 cycles (or 3 cycles if beyond C11). Imaging may be performed sooner if there is clinical concern for progression.
i. Research biopsy to be performed at Cycle 2 Day 1
j. At C1D1, re-staging visits and at time of progression, 1 tube of whole blood will be collected in a 10mL Streck tube for cfDNA analysis. If sample collection is missed for any reason at baseline or at the time of progression then the sample should be drawn at a future appointment.
k. A standard 12-Lead ECG For patients with QTcF ≥481 ms at any time, interrupt study treatment and follow the procedures described in the "Ribociclib Dose Modification section". If treatment is resumed, repeat ECGs 7 days and 14 days after dose resumption (and then as clinically indicated). During subsequent cycles, perform predose ECG for every cycle starting at cycle 6, and predose and 2-4 postdose starting at cycle 9 and every 3rd cycle thereafter.

Table 10-3 Study calendar for Cohort B, Phase II Study

	Screening[a]	Day 1 of each cycle	End of treatment
Ribociclib[b]		X	
Trastuzuman[c]		X	
Informed consent	X		
Inclusion/Exclusion Criteria	X		
Demographics	X		
Medical history	X		
ConMeds	X	X	
Physical exam	X	X	X
AE assessment	X	X	X
Vital signs	X	X	X
Height	X		
Weight	X	X	
Performance status	X	X	X
Labs[d]	X	X	X
PT-INR	X	X	X
Urinalysis/BUN	X	X	X
Lipid Panel	X	X[e]	
ECG	X	X[f]	X
MUGA/ECHO	X	X[g]	
Tumor assessments	X	X[h]	
Pregnancy Test	X		
Hepatitis B and C Test	X		
Research Biopsy	X	X[i]	
Blood for cfDNA[j]	X	X[j]	X
CTC	X		
Survival Follow-up			X

a. Screening Imaging (CT, MRI and ECHO/MUGA) can be done within 28 days of starting protocol therapy. Screening labs should be done within 14 days of starting protocol therapy.
b. Ribociclib is given continuously Days 1-21 of a 21 day cycle except for at Dose Level -2 where Ribociclib is given Day 1-14 of a 21 day cycle.
c. Trastuzumab should be administered within +/- 3 days of the scheduled assessment unless

otherwise noted.

d. Labs to include: hematology, clinical chemistry (including calcium, magnesium, and phosphorus), and liver function tests (albumin, ALT, total protein, alkaline phosphotase, AST, and total bilirubin). Cycle 1 Day 1 labs do not need to re-meet eligibility criteria.
e. Lipid Panel to be drawn on Day 1 every 4 cycles beginning at screening (i.e. screening, then day 1 of cycles 5, 9, 13, etc).
f. ECGs should be done beginning at screening and then, starting with Cycle 2, ECGs should be done every 2 cycles (i.e. screening, and then cycles 2, 4, 6, etc.).
g. ECHO or MUGA using the same cardiac testing modality performed at screening will be performed at the end of every 4th cycle (i.e. Cycle 4, 8, 12, etc.).
h. Tumor Assessments should be done with the same imaging modality as screening and will be performed at screening, the end of cycles 2, 4, 6, and 8 and then at the end of every 3rd cycle (i.e. cycles 11, 14, 17, etc.). End of treatment only required if not done within the previous 2 cycles (or 3 cycles if beyond C11). Imaging may be performed sooner if there is clinical concern for progression.
i. Research biopsy to be performed at Cycle 2 Day 1
j. At C1D1, restaging visits, and at time of progression, 1 tube of whole blood will be collected in a 10mL Streck tube for cfDNA analysis. If sample collection is missed for any reason at baseline or at the time of progression then the sample should be drawn at a future appointment.

Table 10-4 Study Calendar for Cohort C, Phase Ib Study

	Screening[a]	Cycle 1		Cycle 2+		End of treatment
		D1	D15	D1	D15	
Ribociclib[b]		X		X		
Trastuzumab[c]		X	X	X	X	
Fulvestrant		X	X[l]	X		
Informed consent	X					
Inclusion/Exclusion Criteria	X					
Demographics	X					
Medical history	X					
ConMeds	X	X	X	X		
Physical exam	X	X		X		X
AE assessment	X	X	X	X		X
Vital signs	X	X		X		X
Height	X					
Weight	X	X		X		
Performance status	X	X		X		X
Labs[d]	X	X	X	X		X
PT-INR	X	X		X		X
Urinalysis/BUN	X	X		X		X
Lipid Panel	X			X[e]		
ECG[k]	X	X	X[f]	X[f]	[f]	X[f]
MUGA/ECHO	X			X[g]		
Tumor assessments	X			X[h]		X[h]
Pregnancy Test	X					
Hepatitis B and C Test	X					
Research Biopsy	X			X[i]		
Blood for cfDNA[j]	X			X[j]		X
CTC	X					

Survival Follow-up						X

a. Screening Imaging (CT, MRI and ECHO/MUGA) can be done within 28 days of starting protocol therapy. Screening labs should be done within 14 days of starting protocol therapy.
b. Dose level 0: ribociclib is given 400mg PO daily continuously for Days 1-28 of a 28 day cycle. Dose level -1: ribociclib is given at 400mg PO daily for Days 1-21 of 28 day cycle
c. Trastuzumab should be administered within +/- 3 days of the scheduled assessment unless otherwise noted.
d. Labs to include: hematology, clinical chemistry (including calcium, magnesium, and phosphorus), and liver function tests (albumin, ALT, total protein, alkaline phosphotase, AST, and total bilirubin). Cycle 1 Day 1 labs do not need to re-meet eligibility criteria. Labs should be drawn on D1 and D15 of Cycles 1 and 2. Starting in Cycle 3, labs should only be drawn on D1 of each cycle.
e. Lipid Panel to be drawn on Day 1 every 4 cycles beginning at screening (i.e. screening, then day 1 of cycles 5, 9, 13, etc).
f. ECGs performed on C1D1, C1D15, C2D1, C2D15, and then every 8 weeks. ECGs on C1D1 and C1D15 should be done pre-dose and 2-4 hours post-dose.
g. ECHO or MUGA using the same cardiac testing modality performed at screening will be performed at the end of every 3rd cycle (i.e. Cycle 3, 6, 9, etc.).
h. Tumor Assessments should be done with the same imaging modality as screening and will be performed at screening, the end of cycles 2, 4, and 6, and then at the end of every 3rd cycle (i.e. cycles 9, 12, 15, etc.). End of treatment only required if not done within the previous 2 cycles (or 3 cycles if beyond C9). Imaging may be performed sooner if there is clinical concern for progression.
i. Research biopsy to be performed at Cycle 2 Day 1
j. At C1D1, re-staging visits and at time of progression, 1 tube of whole blood will be collected in a 10mL Streck tube for cfDNA analysis. If sample collection is missed for any reason at baseline or at the time of progression then the sample should be drawn at a future appointment.
k. A standard 12-Lead ECG For patients with QTcF ≥481 ms at any time, interrupt study treatment and follow the procedures described in the "Ribociclib Dose Modification section". If treatment is resumed, repeat ECGs 7 days and 14 days after dose resumption (and then as clinically indicated). During subsequent cycles, perform predose ECG for every cycle starting at cycle 6, and predose and 2-4 hour postdose starting at cycle 9 and every 3rd cycle thereafter.
l. Fulvestrant dosing on C1D15 not required for subjects who have received fulvestrant within 6 months prior to starting study.

Table 10-5 Study calendar for Cohort C, Phase II Study

	Screening[a]	Day 1 of each cycle	Day 15 of each cycle	End of treatment
Ribociclib[b]		X		
Trastuzuman[c]		X	X	
Fulvestrant		X	X[d]	
Informed consent	X			
Inclusion/Exclusion Criteria	X			
Demographics	X			
Medical history	X			
ConMeds	X	X	X	
Physical exam	X	X		X
AE assessment	X	X	X	X
Vital signs	X	X		X
Height	X			
Weight	X	X		
Performance status	X	X		X
Labs[e]	X	X	X[e]	X
PT-INR	X	X		X
Urinalysis/BUN	X	X		X
Lipid Panel	X	X[f]		
ECG[l]	X	X[g]	X[g]	X
MUGA/ECHO	X	X[h]		
Tumor assessments	X	X[i]		
Pregnancy Test	X			
Hepatitis B and C Test	X			
Research Biopsy	X	X[j]		
Blood for cfDNA[k]	X	X[k]		X
CTC	X			
Survival Follow-up				X

a. Screening Imaging (CT, MRI and ECHO/MUGA) can be done within 28 days of starting protocol therapy. Screening labs should be done within 14 days of starting protocol therapy.
b. Dosing levels depend on starting dose determined in Phase 1 b patients. See Tables 6-3 and 6-4 for dose details.
c. Trastuzumab should be administered within +/- 3 days of the scheduled assessment unless

otherwise noted.

d. Fulvestrant should be administered on D15 of Cycle 1 only. Subjects who received fulvestrant within 6 months of starting protocol therapy do not require the D15 dose.
e. Labs to include: hematology, clinical chemistry (including calcium, magnesium, and phosphorus), and liver function tests (albumin, ALT, total protein, alkaline phosphatase, AST, and total bilirubin). Cycle 1 Day 1 labs do not need to re-meet eligibility criteria. Labs should be performed on D1 and D15 of Cycles 1 and 2. Starting in Cycle 3, labs only need to be performed on D1 of each cycle.
f. Lipid Panel to be drawn on Day 1 every 4 cycles beginning at screening (i.e. screening, then day 1 of cycles 5, 9, 13, etc).
g. ECGs performed on C1D1, C1D15, C2D1, C2D15, and then every 8 weeks.
h. ECHO or MUGA using the same cardiac testing modality performed at screening will be performed at the end of every 3rd cycle (i.e. Cycle 3, 6, 9, etc.).
i. Tumor Assessments should be done with the same imaging modality as screening and will be performed at screening, the end of cycles 2, 4, and 6, and then at the end of every 3rd cycle (i.e. cycles 9, 12, 15, etc.). End of treatment only required if not done within the previous 2 cycles (or 3 cycles if beyond C9). Imaging may be performed sooner if there is clinical concern for progression.
j. Research biopsy to be performed at Cycle 2 Day 1
k. At C1D1, restaging visits, and at time of progression, 1 tube of whole blood will be collected in a 10mL Streck tube for cfDNA analysis. If sample collection is missed for any reason at baseline or at the time of progression then the sample should be drawn at a future appointment.
l. A standard 12-Lead ECG For patients with QTcF ≥481 ms at any time, interrupt study treatment and follow the procedures described in the "Ribociclib Dose Modification section". If treatment is resumed, repeat ECGs 7 days and 14 days after dose resumption (and then as clinically indicated). During subsequent cycles, perform predose ECG for every cycle starting at cycle 6, and predose and 2-4 hour postdose starting at cycle 9 and every 3rd cycle thereafter.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 2 cycles (6weeks). If patients experience a lack of progression after 4 re-staging examinations (after 8 cycles), the interval for staging will increase to 3 cycles for all patients.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression

prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: 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.

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.

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

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. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a 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 unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In 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. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET

and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

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 participant to be considered in complete clinical response.

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.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) 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 (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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.

11.1.4.2 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 patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 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 patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response 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	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** 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.

11.1.5 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 recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

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 progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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, including the baseline measurements.

11.1.6 Progression and Survival Metrics

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from study entry to the earlier of progression (by RECIST 1.1 or documented evidence of clinical deterioration due to breast cancer) or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.7 Response Review

The study will use the DF/HCC Tumor Imaging Metrics Core (TIMC) for central protocol measurements.

11.2 Other Response Parameters

Objective response rate: Objective response rate (ORR) as defined by RECIST1.1.

Clinical benefit rate: Clinical benefit rate (CBR) is defined as the proportion of patients with a CR, PR, or SD at week 12.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

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

12.1.2 Responsibility for Data Submission

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

12.2 Data Safety Monitoring

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

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

13. STATISTICAL CONSIDERATIONS

Cohort A

Cohort A: Standard 3+3 phase-I design will be utilized in this trial. Briefly, a minimum of 3 evaluable patients will be entered at first dose level (=300 mg ribociclib) and T-DM1 (3.6 mg/kg IV). If 1 out of the first 3 patients enrolled experiences a dose-limiting toxicity (DLT), 3 additional patients will be enrolled to that dose level. If no more than 1 patient in 6 experiences a DLT, dose escalation of ribociclib will continue to next dose-level. If 2 or more patients at any given dose level experience a DLT, dose escalation will stop and the maximum tolerated dose (MTD) will be defined. We will not exceed the R2PD of ribociclib (= 600 mg) single agent. At least 6 patients will be treated at the MTD/R2P2d of ribociclib and T-DMl combination. Once MTD/R2PD is determined there will be a small dose-expansion cohort (N= l5) to confirm safety profile and evaluate preliminary evidence of efficacy. The operating characteristics of the dose escalation portion of this study are shown in the table below, which provides the probability of declaring the MTD for a range of underlying true DLT rates. For example, for a toxicity that occurs in 60% of patients, there is less than 10% probability (0.08) of declaring the MTD.

Table. Probability of Escalation

True underlying DLT rate	10%	20%	30%	40%	50%	60%	70%	80%	90%
Probability of declaring MTD	91%	71%	49%	31%	17%	8%	3%	1%	0.1%
Probability of escalation	9%	29%	51%	69%	83%	92%	97%	99%	99.9%

In the dose-expansion cohort, the following table provides the probability of observing any serious toxicities under various true rates in the general population.

	True rate of serious toxicity:			
Number of patients	5%	10%	15%	20%
15	0.54	0.79	0.91	0.96

Cohort B

This is a single-arm, two stage design to assess the clinical benefit rate (CR, PR, and SD at 24 weeks) with ribociclib in combination with trastuzumab, with an additional safety run-in of 6 patient cohorts and de-escalation design to establish the MTD. Briefly, a minimum of 6 evaluable patients will be entered at the first dose level (see section 5.2). If 2 or more patients experience a dose-limiting toxicity (DLT), 6 additional patients will be enrolled to the next lower dose level. If no more than 1 patient in 6 experiences a DLT, enrollment will continue to the first stage of the efficacy evaluation.

Table. Probability of De-escalation

True underlying DLT rate	10%	20%	30%	40%	50%	60%	70%	80%	90%
Probability of declaring MTD	89%	66%	42%	23%	11%	4%	1%	0.2%	<0.01%
Probability of de-escalation	11%	34%	58%	77%	89%	96%	99%	99.8%	>99.9%

A clinical benefit rate of 45% among patients with advanced breast cancer would be considered worthy of further study. A rate of 24% or less would not be of clinical interest (null hypothesis). In the first stage 20 patients will be enrolled. If 6 or more patients show CR, PR, or SD at 24 weeks, accrual will continue to the second stage where an additional 15 patients will be enrolled. If there are at least 12 patients showing clinical benefit at 24 weeks among the 35 patients, the regimen will be declared worthy of further study. The sample size was chosen to have a high power (90%) and a Type I error rate of no more than 10%.

Biopsies will be mandatory in the phase 2 potion of Cohort B. It is anticipated that 60-70% of patients will have phospho-Rb reduction after one cycle of protocol treatment. Assuming 25 pairs of biopsies will be evaluable for phospho-Rb, a 45% clinical benefit rate and a 60% phospho-Rb reduction rate, we have 80% power to detect the difference of clinical benefit rates between patients with and without phospho-Rb reduction if the clinical benefit rate is 67% among patients with phosphor-Rb reduction and 12% among patients without phosphor-Rb reduction. The following table gives the power of detecting the clinical benefit rate difference among patients with and without phosphor-Rb reduction, assuming clinical benefit rate is 45% and number of paired biopsies evaluable for phospho-Rb is 25 or 30.

# of paired biopsies evaluable for phosphor-Rb	Expected tumor with phosphor-Rb reduction	# of pts with phosphor-Rb reduction	# of pts without phosphor-Rb reduction	CBR among pts with phosphor-Rb reduction	CBR among pts without phosphor-Rb reduction	Power
25	60%	15	10	68%	10%	86%
				67%	12%	80%
				65%	15%	70%
	68%	17	8	64%	5%	88%

				62.5%	8%	79%
				60.5%	12%	66%
30	60%	18	12	70%	7.5%	96%
				65%	15%	80%
				60%	22.5%	52%
	70%	21	9	62%	5%	91%
				60%	10%	76%
				58%	15%	59%

Cohort C

This is a single-arm, two stage design to assess the clinical benefit rate (CR, PR, and SD at 24 weeks) with ribociclib in combination with trastuzumab and fulvestrant. The first 6 patients will also form a "safety run-in" cohort, and will establish the MTD which will be used for the rest of the patients accrued to this cohort. Briefly, a minimum of 6 evaluable patients will be entered at the first dose level (see section 5.2). If 2 or more patients experience a dose-limiting toxicity (DLT), 6 additional patients will be enrolled to the next lower dose level. If no more than 1 patient in 6 experiences a DLT, enrollment will continue to the phase 2 portion of the study. Note, all patients treated at the RP2D (both in the safety run-in and phase 2 portion) will be included in efficacy analysis.

Table. Probability of De-escalation

True underlying DLT rate	10%	20%	30%	40%	50%	60%	70%	80%	90%
Probability of declaring MTD	89%	66%	42%	23%	11%	4%	1%	0.2%	<0.01%
Probability of de-escalation	11%	34%	58%	77%	89%	96%	99%	99.8%	>99.9%

A clinical benefit rate of **40%** among patients with **HER2-positive, ER-positive** advanced breast cancer would be considered of clinical interest for a regimen comprising of **fulvestrant, trastuzumab, and ribociclib**. A rate of **20%** or less would not be of clinical interest (null hypothesis). In the first stage **19** patients will be enrolled (including those enrolled to the safety run-in phase). If **4** or more patients show CR, PR, or SD at 24 weeks, accrual will continue to the second stage where an additional **17** patients will be enrolled. If there are at least **11** patients showing clinical benefit at 24 weeks among the **36** patients, the regimen will be declared worthy of further study. The sample size was chosen to have a high power (90%) and a Type I error rate of no more than 10% **using a Simon Minimax two-stage design**.

NB – the null hypothesis rate of 20% is lower than that for cohort B (24%). The clinical benefit rate of 24% used for cohort B was taken from a clinical trial of patients with treatment-refractory HER2-positive disease (Blackwell JCO 2010). In this study, the regimen of trastuzumab and lapatinib yielded a clinical benefit rate of 24%. However, patients in this study had not been previously treated with the highly effective anti-HER2 agents T-DM1 and pertuzumab, as they were not available at that time. In Cohort C, however, all patients must have received prior pertuzumab and T-DM1, as well as standard therapies such as trastuzumab. There is no trial data on the efficacy of systemic therapy in such patients, and thus the null hypothesis for Cohort C was chosen to reflect a slight reduction in the CBR seen in the Blackwell study, given the differences in the patient populations in terms of prior therapy.

Biopsies will be mandatory in the phase 2 portion of Cohort B. It is anticipated that 60-70% of patients will have phospho-Rb reduction after one cycle of protocol treatment. Assuming 25 pairs of biopsies will be evaluable for phospho-Rb, a 40% clinical benefit rate and a 60% phospho-Rb reduction rate, we have 89% power to detect the difference of clinical benefit rates between patients with and without phospho-Rb reduction if the clinical benefit rate is 60% among patients with phospho-Rb reduction and 10% among patients without phospho-Rb reduction. The following table gives the power of detecting the clinical benefit rate difference among patients with and without phosphor-Rb reduction, assuming clinical benefit rate is 40% and number of paired biopsies evaluable for phospho-

Rb is 25 or 30. The power calculation was conducted using McNemar's test with 1-sided alpha of 0.2.

# of paired biopsies evaluable for phospho-Rb	Expected tumor with phospho-Rb reduction	# of pts with phospho-Rb reduction	# of pts without phospho-Rb reduction	CBR among pts with phospho-Rb reduction	CBR among pts without phospho-Rb reduction	Power
25	60%	15	10	60%	10%	89%
				53%	20%	82%
				47%	30%	77%
	68%	17	8	53%	13%	96%
				47%	25%	93%
				41%	38%	90%
30	60%	18	12	61%	8%	93%
				56%	17%	88%
				50%	33%	84%
	70%	21	9	52%	11%	99%
				48%	22%	98%
				43%	33%	96%

13.1 Interim Monitoring Plan

Refer to Section 5.7, Section 10.

13.2 Analysis of Primary Endpoints

Cohort A

Maximum tolerated dose (MTD) and/or recommended phase2 dose (RP2D) of ribociclib in combination with T-DM1

Please refer to Section 1.1.

Cohort B and Cohort C

Clinical benefit rate (CBR):

CBR is defined as the proportion of patients with a complete response (CR) or partial response (PR), or with stable disease (SD) at week 24 by RECIST 1.1 criteria. CBR will be reported with 90% confidence interval, adjusting for two-stage design using the method from Atkinson and Brown.

13.3 Analysis of Secondary Endpoints

Cohort A

Plasma concentrations of ribociclib

Please refer to Section 9.3.

Objective response rate (ORR):

ORR is defined as the proportion of patients with complete response or partial response by RECIST 1.1 criteria. ORR will be reported with 90% confidence interval using the exact binomial method.

Progression-free survival (PFS):
PFS is defined as the time from study entry to the first documented evidence of disease progression by RECIST 1.1 or death from any cause whichever occurs first. For this analysis, participants will also be considered to have progressed if they discontinue treatment with documented evidence of clinical deterioration due to breast cancer. Participants alive without disease progression will be censored at the time of last disease evaluation. PFS will be summarized using Kaplan-Meier survival method.

Biomarkers:
All analyses of correlative scientific endpoints are exploratory and hypothesis-generating. Any promising findings will be further tested in future studies.

Cohort B and Cohort C
Objective response rate (ORR):
ORR is defined as the proportion of patients with complete response or partial response by RECIST 1.1 criteria.

Progression-free survival (PFS):
PFS is defined as the time from study entry to the first documented evidence of disease progression by RECIST 1.1 or death from any cause whichever occurs first. For this analysis, participants will also be considered to have progressed if they discontinue treatment with documented evidence of clinical deterioration due to breast cancer. Participants alive without disease progression will be censored at the time of last disease evaluation. PFS will be summarized using Kaplan-Meier survival method.

Overall survival (OS):

OS is defined as the time from study entry until death from any cause. For participants who do not die, time to death will be censored at the time of last contact. OS will be summarized using a Kaplan-Meier survival curve.

Adverse Events (AE):
All participants will be evaluable for toxicity from the time of their first treatment with any study agent. Toxicity will be graded according to NCI CTCAE, Version 4.0. Results will be tabulated.

Biomarkers:
Phospho-Rb will be evaluated at baseline at after one cycle of protocol treatment. We will then evaluate whether the reduction of phospho-Rb is associated with clinical benefit using Fisher's exact method. All analyses of correlative scientific endpoints are exploratory and hypothesis-generating. Any promising findings will be further tested in future studies.

13.4 Reporting and Exclusions

13.4.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment.

13.4.2 Evaluation of the Primary Efficacy Endpoint

All patients who receive at least one dose of protocol therapy are evaluable for clinical benefit.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B CONCOMITANT MEDICATIONS

In general, the use of any concomitant medication deemed necessary for the care of the patient is permitted in this study, except as specifically prohibited below. Combination administration of study drugs could result in drug-drug interactions (DDI) that could potentially lead to reduced activity or enhanced toxicity of the concomitant medication and/or ribociclib.

The following lists are not comprehensive and are only meant to be used as a guide. The lists are based on the Oncology Clinical Pharmacology guidance, Drug-Drug Interaction Interaction and Co-medication Consideration (v05 release date: 2015), which was compiled from the Indiana University School of Medicine's P450 Drug Interaction Table (http://medicine.iupui.edu/clinpharm/ddis/main-table/) and supplemented with the FDA Draft Guidance for Industry, Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling (February 2012) (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf), and the University of Washington's Drug Interaction Database (http://www.druginteractioninfo.org/). For current lists of medications that may cause QT prolongation and/or torsades de pointes (TdP), refer to the CredibleMeds® website (www.qtdrugs.org

TABLE 1. LIST OF PROHIBITED MEDICATIONS DURING STUDY DRUG TREATMENT

Category	Drug Name
Strong CYP3A4/5 inhibitors	voriconazoleBoceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, eltegravir/ritonavir, grapefruit juice, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycin, voriconazole
Strong CYP3A4/5 inducers	Avasimibe[2,3], carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin)[3], St. John's wort (hypericum perforatum)[3]
CYP3A4/5 substrates with NTI[1]	terfenadineAlfentanil, apixaban (doses >2.5 mg only), aprepitant, astemizole, cisapride, cyclosporine, diergotamine, dihydroergotamine, ergotamine, fentanyl, lovastatin, nicardipine, nisoldipine, pimozide, quinidine, rivaroxaban, simvastatin, sirolimus, tacrolimus, terfenadine, thioridazineAlfentanil
Medications with a known risk for QT prolongation[4]	vavdetanibAmiodarone, anagrelide, arsenic trioxide, astemizole, azithromycin, bepridil, chloroquine, chlorpromazine, cilostazol, ciprofloxacin, cisapride, citalopram, clarithromycin, disopyramide, dofetilide, domperidone, donepezil, dronedarone, droperidol, erythromycin, escitalopram, flecainide, fluconazole, halofantrine, haloperidol, ibutilide, levofloxacin, levomethadyl, mesoridazine, methadone, moxifloxacin, ondansetron (i.v. only), pentamidine, pimozide, probucol, procainamide, propofol, quinidine, sevoflurane, sotalol, sparfloxacin, sulpiride, terfenadine, thioridazine, vandetanib, venlafaxine
Herbal preparations/ medications	Herbal preparations/medications are prohibited throughout the study. These herbal medications include, but are not limited to: St. John's wort, Kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 7 days prior to first dose of study drug.
Other investigational and antineoplastic therapies	Other investigational therapies must not be used while the patient is on the study. Anticancer therapy (chemotherapy, biologic or radiation therapy, and surgery) other than the study treatments must not be given to patients while the patient is on the study medication. If such agents are required for a patient then the patient must be discontinued study drug.

[1] NTI = narrow therapeutic index drugs whose exposure-response indicates that increases in their exposure levels by the concomitant use of potent inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).

[2] Herbal product

[3] P-gp inducer

[4] Source: www.qtdrugs.org (as of Apr 7, 2015)

TABLE 2. LIST OF MEDICATIONS TO BE USED WITH CAUTION DURING STUDY DRUG TREATMENT

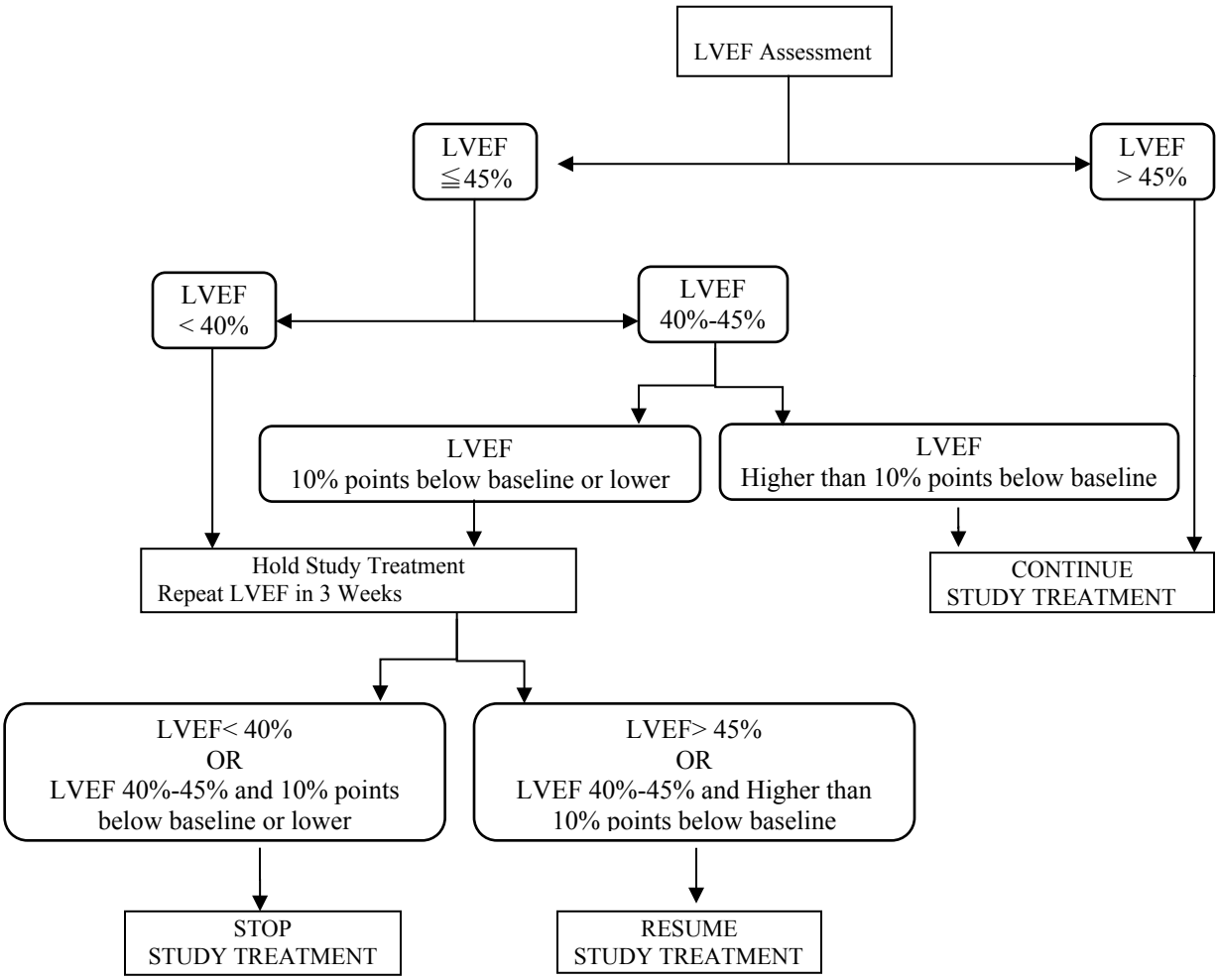
Category	Drug Name
Moderate CYP3A4/5 inhibitors	Amprenavir, atazanavir, casopitant, cimetidine, darunavir, diltiazem, fosamprenavir, lomitapide, netupitant, tofisopam, verapamil
Moderate CYP3A4/5 inducers	Bosentan, efavirenz, etravirine, genistein, lersivirine, modafinil, nafcillin, talviraline
Sensitive CYP3A4/5 substrates[1]	Alpha-dihydroergocryptine, almorexant, alpaviroc, apixaban (doses < 2.5 mg only), atazanavir, atorvastatin, avanafil, bosutinib, brecanavir, brotizolam, budesonide, buspirone, capravirine, casopitant, darifenacin, darunavir, ebastine, eletriptan, eplerenone, felodipine, fluticasone, ivacaftor, lomitapide, lumefantrine, lurasidone, maraviroc, midazolam, perospirone, quetiapine, ridaforolimus, sildenafil, ticagrelor, tilidine, tolvaptan, triazolam, vardenafil, vicriviroc, voclosporin
Strong BSEP inhibitors	Bosentan, fusidate, glibenclamide, sulindac, troglitazone (TGZ-sulfate)
Medications that carry a possible risk for QT prolongation[2]	Alfuzosin, apomorphine, aripiprazole, atazanavir, atomoxetine, bedaquiline, clozapine, dexmedetomidine, dolasetron, eribulin, famotidine, felbamate, fingolimod, foscarnet, gatifloxacin, gemifloxacin, granisetron, iloperidone, isradipine, lithium, mirabegron, mirtazapine, moexipril, norfloxacin, ofloxacin, olanzapine, ondansetron (p.o. only at 4 mg or 8 mg), oxytocin, paliperidone, pasireotide, pipamperone, promethazine, quetiapine, ranolazine, rilpivirine, risperidone, roxithromycin, sertindole, telavancin, tetrabenazine, tizanidine, tolterodine, vardenafil, ziprasidone
MATE1 and OCT2 substrates[3]	Acyclovir, amantadine, amiloride, cephalexin, cephradine, cimetidine, famotidine, fexofenadine, memantine, metformin (also a substrate for OCT1, MATE1, and MATE2K), pindolol, procainamide, ranitidine, and varencicline
BCRP substrates	Rosuvastatin, sulfasalazine

[1] Sensitive substrates: Drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a potent inhibitor.

[2] Source: www.qtdrugs.org (as of Apr 7, 2015)

[3] Source: FDA Draft Guidance for Industry, Drug Interaction Studies – Study Design, Data Analysis, and implications for Dosing and Labeling (February 2012) and Yonezawa and Inui (2011) Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics. Br J Pharmacology 164:1817-25

APPENDIX C: ALGORITHM FOR CONTINUATION AND DISCONTINUATION FOR LEFT VENTRICULAR DYSFUNCTION



LVEF= left ventricular ejection fraction
Study Treatment : Cohort A: Ribociclib and T-DM1; Cohort B: Ribociclib and trastuzumab.

Discontinue study treatment for symptomatic CHF.

Patients for whom study treatment was permanently discontinued due to a drop in LVEF should continue to have LVEF assessments repeated as clinically indicated, with a maximum interval between LVEF assessments of 3 months, until the LVEF values return to >50% or 1 year after the Treatment Discontinuation Visit, whichever comes first. Thereafter, LVEF assessment will be performed annually for up to 3 years after the Treatment Discontinuation Visit.

APPENDIX D: NEW YORK HEART ASSOCIATION CARDIAC DISEASE CLASSIFICATION

The New York Heart Association (NYHA) Cardiac Disease Classification provides a functional and therapeutic classification for the prescription of physical activity for cardiac subjects. Based on NYHA definitions, subjects are to be classified as follows:

Class	Definition
Class I	Subjects with no limitation of activities; they suffer no symptoms from ordinary activities.
Class II	Subjects with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.
Class III	Subjects with marked limitation of activity; they are comfortable only at rest.
Class IV	Subjects who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

APPENDIX E TISSUE ACQUISITION GUIDELINES

For biopsies of soft tissue, liver, breast, etc:

Attempts should be made to obtain 3-6 18 gauge core biopsies if deemed feasible and safe by the physician performing the procedure. Cases where less tissue is obtained will still be included in the correlative studies. The cores should be processed as follows:

Core 1: Snap-frozen in OCT
Core 2: Fixed in 10% neutral buffered formalin
Core 3: Snap-frozen in OCT
Core 4: Fixed in 10% neutral buffered formalin
Core 5: Snap-frozen in OCT
Core 6: Snap-frozen in OCT

Snap-freezing guidelines:

- After the biopsy is performed, place tissue on a sterile gauze
- Use forceps to separate tissue cores from one another
- Place maximum of 2 cores in a cassette (if 3 cores frozen, place 2 cores in first cassette and 1 core in second cassette).
- Fill histology cassettes with OCT. Completely cover the tissue with OCT and limit bubble formation
- Place cassettes on dry ice and prepare for transport by limiting OCT leakage
- Return samples to lab and complete freezing of samples in OCT with dry ice (about 10 minutes freezing time)
- Once samples are frozen, place in plastic bag; label bag with unique specimen identification number, protocol number, patient study registration number, date of biopsy, and site of biopsy.
- Deliver samples to TRL, MGH (cohort A), or Shom Goel, DFCI (cohort B)
- Store in -80C freezer

Fixation in 10% neutral buffered formalin guidelines:

- After the biopsy is performed, place tissue on a sterile gauze
- Use forceps to separate tissue cores from one another
- Place cores in a 5mL vial containing 3mL of 10% neutral buffered formalin
- Ensure that cores are completely submerged in the formalin
- Deliver samples to TRL, MGH (cohort A), or Shom Goel, DFCI (cohort B)

For biopsies of skin and chest wall:

Aim to obtain 2 x 5mm punch biopsies. The first should be frozen in OCT and the second fixed in 10% NBF as described above.

Fine Needle Aspiration Samples
For FNA samples there is a goal to perform 3 passes.

Pass 1: evacuated, rinse directly in 10-20mL of RPMI to prepare a cell block
Pass 2: evacuate and rinse directly into 2mL of room temperature trizol for DNA analysis
Pass 3: evacuated, rinse directly in 10-20mL of RPMI to prepare a cell block which should be snap frozen in an EtOH/dry ice bath or in liquid N2.

For Effusions and Ascites

Fluid sample should be split into two equal aliquots. One aliquot should be spun down into a pellet and snap frozen in an ETOH/dry ice bath or in liquid N2. The second aliquot should be fixed and processed as a standard cell block. 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.

All samples will be anonymized by assigning a unique specimen ID number prior to use. Histopathologic review will be performed at DFCI/BWH or MGH 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 actually processed.

Fresh Tissue Shipping Procedures

Cohort A

Attn: John Iafrate,
Translational Research Lab,
Massachusetts General Hospital,
55 Fruit St, Boston, MA, 02114
Ph: 617 726-2967
Fax: 617-726-7474

Cohort B and Cohort C

Please ship frozen specimens over-night on dry ice to the following:

Attention: Shom Goel
450 Brookline Avenue, Smith 934
Boston, MA 02215
Ph: 617-632-5967
Fax: 617-632-3550

Email Shom Goel (sgoel@partners.org), April Watts (chins_watt@dfci.harvard.edu), and the current Dana-Farber CRC with the sample information and tracking information before shipping specimens.

APPENDIX F GUIDELINES FOR USE OF STRECK TUBES FOR LABS OUTSIDE OF DFCI

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

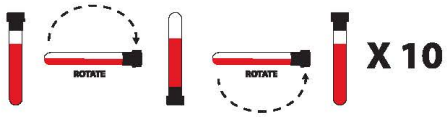
1. When stored at 18-30°C, unused Cell-Free DNA BCT is stable through expiration date.
2. Do not freeze unfilled Cell-Free DNA BCT. Proper insulation may be required for shipment during extreme temperature conditions.
3. Blood samples collected in Cell-Free DNA BCT for cf-DNA analysis are stable for 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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3. Norton, S.E., Lechner, J.M., Williams, T., and Fernando, M.R., (2013). "A Stabilizing Reagent Prevents Cell-free DNA Contamination by Cellular DNA in Plasma during Blood Sample Storage and Shipping as Determined by Digital PCR." *Clinical Biochemistry*, 2013; 46: 1561-1565.
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6. Clinical and Laboratory Standards Institute. H3-A6, *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard-Sixth Edition.*

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.

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