DF/HCC Protocol: 17-024

TITLE: A Phase 2 Study of Abemaciclib for Patients with Retinoblastoma-Positive, Triple Negative Metastatic Breast Cancer

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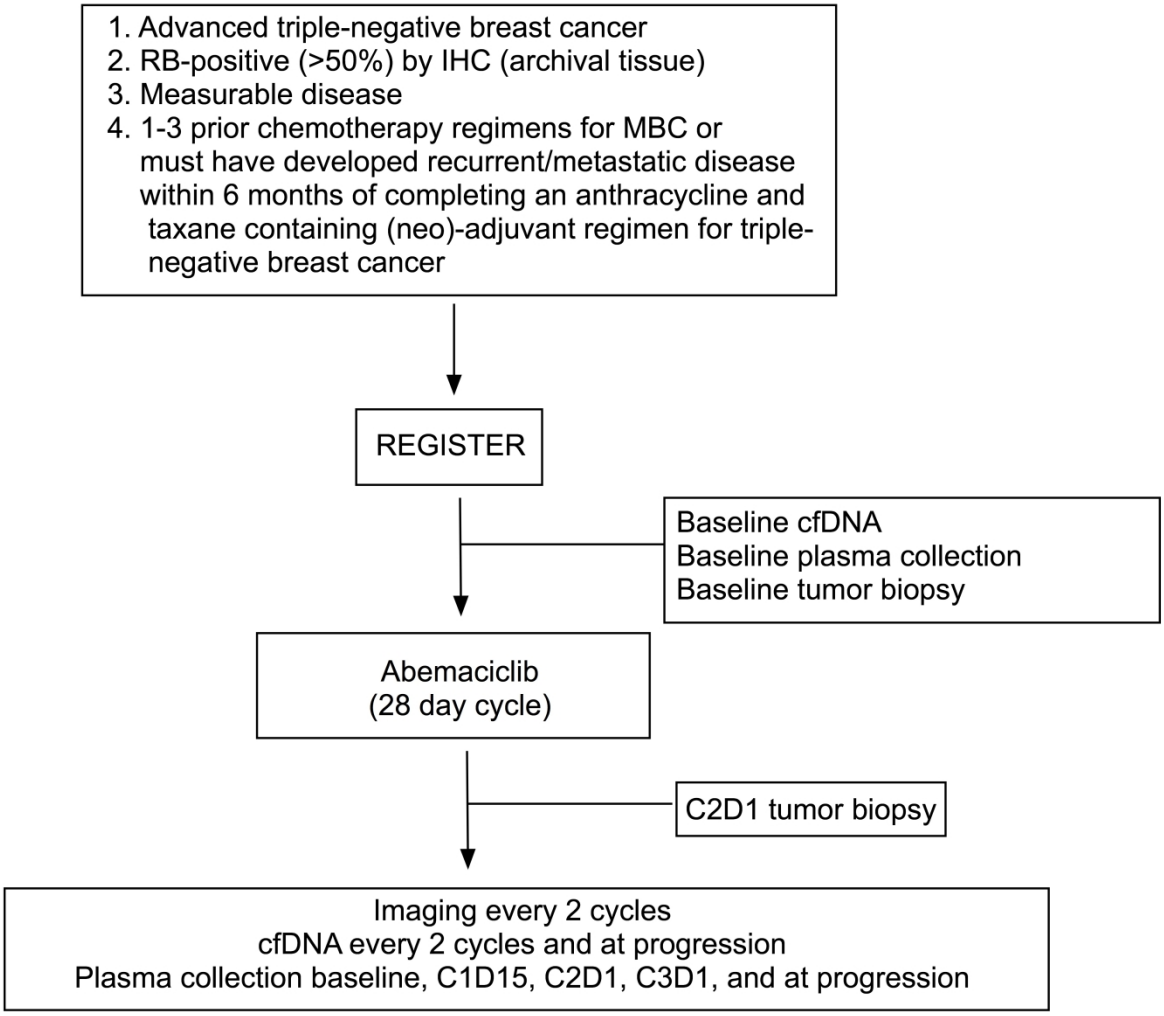
Protocol Type / Version # / Version Date: Amendment / Version 9 / November 11, 2019

SCHEMA


1. Advanced triple-negative breast cancer
2. RB-positive (>50%) by IHC (archival tissue)
3. Measurable disease
4. 1-3 prior chemotherapy regimens for MBC or must have developed recurrent/metastatic disease within 6 months of completing an anthracycline and taxane containing (neo)-adjuvant regimen for triple-negative breast cancer
REGISTER
Baseline cfDNA
Baseline plasma collection
Baseline tumor biopsy
Abemaciclib
(28 day cycle)
C2D1 tumor biopsy
Imaging every 2 cycles
cfDNA every 2 cycles and at progression
Plasma collection baseline, C1D15, C2D1, C3D1, and at progression

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1. OBJECTIVES

1.1 Study Design

This is a single-arm, two-stage phase 2 study to evaluate the response to abemaciclib monotherapy in patients with Rb-positive, triple negative metastatic breast cancer. The first stage of the study includes 13 patients with Rb-positive, triple negative metastatic breast cancer. If in the first stage there is at least one response, accrual will continue to the second stage where an additional 25 patients will be enrolled. If there are at least 4 responses among the 38 patients, the regimen would be considered to be worthy of further study.

1.2 Primary Objectives

To evaluate the efficacy of abemaciclib, as defined by objective response rate (ORR) in patients with Rb-positive triple-negative metastatic breast cancer (ORR as confirmed Complete Response (CR) or Partial Response (PR) per Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1).

1.3 Secondary objectives

1.3.1 To evaluate abemaciclib with respect to each of the following:

1) Progression-Free Survival (PFS)
2) Overall Survival (OS)
3) Disease control rate (DCR)
4) Clinical benefit rate (CBR)

To detect if there is a difference in clinical outcomes between patients with and without phospho-Rb reduction.

1.4 Exploratory objectives

To explore additional potential biomarkers in blood and tumor tissue samples that might predict response or resistance to abemaciclib.

To determine the association between genomic alterations in tumor DNA (mutations, copy number alterations, mutational burden) and RB expression and other tissue biomarkers by immunohistochemistry as well as response to abemaciclib.

To determine if there are any changes in the T-cell phenotype during exposure to abemaciclib.

To explore changes in the microbiome induced by therapy comparing stool samples collected at

baseline, during treatment, and at the time of progression. Changes in microbiome will be compared to other trial endpoints.

2. BACKGROUND

2.1 Triple-Negative Breast Cancer

Breast cancer remains a major cause of cancer death for women globally. Indeed in the USA alone, more than 39,000 women succumbed to the disease is 2013 [1]. Breast cancer is comprised of a number of different subtypes, and the triple negative subtype (TNBC) currently presents the most significant clinical challenges [2] [3]. At present, the only available systemic therapies for patients with advanced TNBC are cytotoxic chemotherapies, and there are no targeted or biologic therapies approved to treat this disease [4].

Unfortunately, there are currently no therapeutic options for patients with advanced TNBC that has progressed after several lines of cytotoxic chemotherapy. Therefore, although TNBC constitutes approximately 20% of all breast cancers, it is the cause of almost half of all breast cancer deaths. Novel approaches to treating the disease are clearly needed.

Recently there has been increasing attention on the use of cell-cycle targeting agents in the treatment of breast cancer. In particular, inhibitors of the cyclin-dependent kinases CDK4 and CDK6 (CDK4/6 inhibitors) are the subjects of great interest, due to the fact that mammary epithelial cells (particularly those of the luminal lineage) are thought to depend on these CDKs for proliferation. In keeping with this, recent trials have confirmed striking efficacy of CDK4/6 inhibitors in patients with estrogen-receptor positive breast cancers [5].

CDK4/6 inhibitors act by inhibiting the phosphorylation of the retinoblastoma tumor suppressor protein (RB). This in turn mitigates the expression of genes under the control of the E2F family of transcription factors, halting progression of tumor cells from the G1 to S phase of the cell cycle. As such, tumors that lack a functional RB protein (whether through gene deletion, mutation, or epigenetic silencing) are predicted to cycle in a CDK4/6-independent manner and thus be inert to the effects of CDK4/6 inhibition [6].

Traditionally, TNBC is associated with loss of function of RB and as such TNBC is not classically considered as a good candidate for response to CDK4/6 inhibition. However, it is clear that there is a subset of TNBC that retains intact RB protein and function. Depending on the methodology used, it is estimated that the rate of Rb loss in TNBC stands between 50-75% [6]. Therefore, there is potential that the remaining TNBC's which retain RB function may exercise cell cycling in an RB-dependent fashion, and that CDK4/6 inhibitors could be an effective treatment strategy for these tumors.

Gene expression analysis from cohorts of TNBC's have identified multiple different TNBC subtypes [7]. Notably, one subtype (the "Luminal Androgen Receptor" (LAR) subtype), shows a strong androgen receptor driven pattern of gene expression, and LAR tumors typically express RB [8]. This group includes somewhere between 10-12% of TNBCs [9]. Recently, Traina et al presented results of a phase 2 study of an androgen receptor inhibitor in patients with

AR-positive metastatic TNBC (i.e. predominantly tumors of the LAR subtype). They observed an 8% response rate and a 16-week clinical benefit rate of 35% [9]. Given that this subset of TNBCs is typically RB-positive, there is a strong biological rationale to suggest that they might be susceptible to CDK4/6 inhibition. Furthermore, recent preclinical work suggests that triple-negative cell lines of both the LAR and mesenchymal-stem like (MSL) subsets are sensitive to CDK4/6 inhibition in vitro [10]. Collectively, the LAR and MSL subtypes comprise approximately 20% of all TNBCs.

Given the rationale for determining the effects of CDK4/6 inhibitors in TNBC with intact RB function, it is important to try and determine RB functionality in TNBCs. At present, there is no standardized way to do this. Various research groups have explored RB expression and/or functionality on TNBCs in the past. One approach has been to use immunohistochemistry (IHC) to the RB protein itself. Using this method, RB is detected in approximately 30-40% of all TNBCs [11, 12], using a definition of positive as "strong nuclear staining". Another has been to measure tumor gene expression and apply signatures of "RB loss". Interestingly, in at least one study such a signature correlated with the presence of loss of heterozygosity (LOH) for the RB locus, but did not correlate well with the presence of RB by IHC [13].

Notwithstanding these studies, there is currently limited data to support a particular method of determining RB functionality in TNBCs, and there is even less data to suggest that one modality would best predict a response to a CDK4/6 inhibitor. Knudsen's laboratory has shown in a small number of TNBC explants that the presence of RB by IHC correlated with sensitivity to CDK4/6 inhibition [14], and given the relative ease of this assay and the ability to apply it in real time to clinical care, this would appear to be a reasonable surrogate for RB functionality in TNBCs.

In the present study, we have elected to use immunohistochemistry to screen tumors for the presence of the Rb protein. Immunohistochemistry has the following advantages:

- RB presence by IHC has been shown to correlate with sensitivity to CDK4/6 inhibitors in TNBC pre-clinically (ref 14)
- Data describing RB-positivity rates by IHC are available from other cohorts (see above)
- The assay can be performed rapidly and reproducibly

To optimize RB staining, we have worked with Dr. Joseph Geradts (pathologist, BWH), who has extensive experience in the study of RB in tumors by immunohistochemistry. We have elected to use the RB antibody clone G3-245 as this has been shown to yield reproducible, reliable RB staining in tissue sections when performed in the BWH IHC laboratory. Having selected this clone, we reviewed the RB staining characteristics (using clone G3-245) of a cohort of 180 TNBCs in a tissue microarray curated by Dr. Nadine Tung and Dr. Stuart Schnitt at the BIDMC, with each case being represented on the microarray in triplicate. The results of this analysis have been formally presented (Patel et al, to ASCO 2017). Tumors were divided into 4 categories: 0% of tumor cells stained; 1-10% of tumor cells stained; 10-50% of tumor cells stained; >50% of tumor cells stained. Approximately 50% of tumors showed >10% of tumor cells positively stained.

We initially chose a cutoff of >10% to define RB positivity for the following reasons:

- The percentage of RB positive tumors by IHC using this cutoff is similar to that seen in previous cohorts.
- This cut off separated tumors into two clearly distinct cohorts with statistically different rates of Androgen Receptor positivity, tumor grade, and patient age (unpublished data). These are all features reported to be more common in RB-functional tumors when compared to RB non-functional tumors.

However, after reviewing the RB-positivity rates of the first 18 patients the RB positivity rate was higher than that observed in the microarray (94% vs 50%). Two pathologists reviewed the stained sections for these cases and compared them to the initial microarray used to define RB cutoffs (Patel et al, ASCO 2017) and observed no differences in scoring techniques.

The higher than expected rate of RB-positivity in the first 18 pre-screened metastatic patients might relate to differences in the populations studied in the initial microarray and our clinical study:

- In the first 18 clinical cases, some of the archival primary tumors tested were of histologic subtypes expected to show high rates of RB expression (e.g. lobular carcinomas). Although these are typically ER-positive, patients with such cancers would still be eligible for the study as long as the most recent tumor tissue specimen from that patient had previously tested triple-negative (whether the primary in question nor a subsequent metastatic biopsy). These archival specimens, would be more likely to show Rb-expression than those in the microarray, which were all primary triple-negative ductal carcinomas.
- The microarray used to define staining cutoffs was enriched for patients with BRCA mutations, which showed a lower rate of RB expression than tumors from BRCA wild-type patients (Patel et al ASCO 2017). Indeed, the RB-positivity rate in BRCA wild-type tumors in the microarray was approximately 60 percent, rather than 50 percent as seen in the entire microarray population. Our initial 18 pre-screened patients were not enriched for BRCA mutation carriers, and might thus be expected to show a rate of RB-positivity higher than 50 percent.

The two reasons described above explain to some extent why the RB-positivity rate in our initial cohort was higher than 50 percent, as observed in the microarray. However, they are unlikely to explain the positivity rate as high as 94 percent. We thus also reviewed each case carefully to determine whether RB staining in our initial trial cohort might be "overcalling" RB-positivity. On review with pathologists (Dr. Geradts and Dr. Schnitt), we noted of the initial 18 cases pre-screened, those with between 11-25% of tumor cells stained (i.e. in the lower range of "positive") all showed very weak staining. Those with strong staining typically showed rates of staining of >50% of tumor cells. Given this, and the uncontrolled pre-analytic variables influencing immunohistochemical stains, we have elected to alter our definition of RB-positive from " >10% of tumor cells staining" to " >50% of tumor cells staining". If this definition were applied to the initial microarray, the rate of RB positivity would be 42 percent amongst all TNBCs, and the rate in our initial cohort of 18 pre-screened patients would be 72%. Given the previously described differences in populations between the microarray and out clinical cohort, we believe that this cutoff represents a more reasonable choice to define "RB-positivity" in this

study.

Any patients enrolled on this trial prior to the RB positivity change from > 10% to > 50% being IRB approved and activated may remain on-study and continue treatment with Abemaciclib per protocol.

2.2 Abemaciclib

Abemaciclib is a selective and potent small molecule inhibitor of CDK4 and CDK6. Abemaciclib demonstrates suitable physical and pharmacokinetic (PK) properties, an acceptable toxicity profile in nonclinical species, and antitumor activity in multiple mouse models of human cancer. Abemaciclib inhibits tumor growth in multiple human xenograft models including, but not limited to, the following tumor types: 1) colorectal cancer, 2) glioblastoma multiforme, 3) acute myeloid leukemia, 4) non-small cell lung cancer, and 5) mantle cell lymphoma. The safety of abemaciclib (LY2835219) has been established in a phase 1, single-arm, dose-escalation study in adult female patients with metastatic breast cancer. The most common grade 3 adverse events (greater than 5% incidence) were neutropenia (33%), leukopenia (22%), abdominal pain (11%), diarrhea (6%) and fatigue (6%). Based on pharmacokinetic data, the recommended phase 2 dose was 200 mg po bid. Recently the results of the MONARCH-1 study were presented. In this study, women with advanced, ER-positive breast cancer that had progressed after 1 or 2 lines of systemic chemotherapy were treated with abemaciclib monotherapy. Strikingly, a 19.5% response rate was observed in this moderately pre-treated population (Dickler et al, ASCO 2016). This data suggests that abemaciclib can have meaningful clinical activity in RB-proficient tumors even in the context of chemotherapy resistance.

The present study is a two-stage single arm phase 2 study of abemaciclib monotherapy in patients with metastatic RB-positive TNBC.The investigators will perform this study in compliance with the protocol, good clinical practice (GCP) and International Conference on Harmonization (ICH) guidelines, and applicable regulatory requirements.

Formally, abemaciclib refers to the free base, whereas LSN2813542 refers to abemaciclib mesylate; however, abemaciclib has been used for uniformity throughout this protocol, except when important for experimental clarity.

More information about the known and expected benefits, risks, and reasonably anticipated adverse events (AEs) of abemaciclib may be found in the Investigator's Brochure (IB). Information on AEs expected to be related to the investigational product may be found in Section 7 (Development Core Safety Information) of the IB. Information on serious adverse events (SAEs) expected in the study population independent of drug exposure and that will be assessed by the sponsor in aggregate, periodically during the course of the study, may be found in Section 6 (Effects in Humans) of the IB.

2.3 Rationale

In cancer cells, genes that normally control the cell cycle are often dysregulated, leading to uncontrolled cellular proliferation [15]. The cyclin D-CDK4/6 holoenzyme controls G1 restriction point via phosphorylation and deactivation of RB [16].

The clinical development of CDK4/6 inhibitors in breast cancer has largely been restricted to patients with ER-positive tumors, as the majority of TNBC's is expected to lack functional RB and are thus not likely to respond to pharmacologic inhibition of CDK4/6. However, as described above a proportion of TNBCs are RB-proficient and in preclinical models have been shown to respond to CDK4/6 inhibition. The present study aims to determine the clinical efficacy and tolerability of abemaciclib in patients with RB-positive, advanced TNBC.

2.4 Correlative Studies Background

In this study, correlative scientific aims will be addressed by performing analysis of both archival patient specimens and specimens obtained during the course of the study. The overarching goal of these studies is to explore possible predictors of response to abemaciclib amongst RB-positive TNBC's. Furthermore, studies will seek evidence of a pharmacodynamic response to abemaciclib therapy (through a tumor biopsy obtained on study) to determine if this correlates with response to therapy.

The main candidate biomarker of tumor sensitivity to CDK4/6 inhibitors is the presence of intact, functional Rb [6]. At the time of screening patients for eligibility, therefore, we will perform immunohistochemistry to identify RB status on archival tumor tissue. Only patients that are RB-positive (greater than 50% of tumor cells staining positive) will be eligible for the study.

Other than RB, there are no validated biomarkers for predicting response/resistance to CDK4/6 inhibitors in breast cancer. Candidate markers of response include amplification of cyclin D1 and loss of the CDK4/6 inhibitor p16 [5], which are thought to render tumors more dependent on the CDK4/6 pathway. Conversely, possible predictors of resistance include amplification of cyclin E1/E2, overexpression of cyclin E, mutations in TP53, and heightened activity of phosphoinositide-dependent kinase 1. Finally, a failure of CDK4/6 inhibition to adequately achieve suppression of RB-phosphorylation, even in the presence of intact RB, is also predicted to be associated with a lack of response to therapy.

In this study, patients with biopsy-accessible disease will be required to undergo tumor biopsy prior to initiating therapy. In addition, patients will undergo a second biopsy after completion of 1 cycle (28 days) of abemaciclib therapy. Analysis of pre-treatment biopsies (or archival tissue when pre-treatment biopsies are not available) will be performed to determine correlations with the predictive biomarkers listed above (and others) and response. Comparison of RB phosphorylation between baseline and C2D1 biopsies will be used to seek evidence of pharmacodynamic effect and explore its association with response. We will also perform exploratory genomic studies (whole exome sequencing) on the tumor tissue, as well as studies on fresh tumor tissue obtained at baseline aimed at determining therapeutic combinations that enhance the effects of CDK4/6 inhibitors in TNBC.

Whole blood will be collected at the time of study entry for circulating tumor DNA (ctDNA).

From this sample, ctDNA will undergo whole exome sequencing (WES) in exploratory genomic studies to determine novel predictors of response to abemaciclib in TNBC. Further details of all correlative studies are provided in section 9 of the protocol.

The primary goal of this study is to determine the clinical efficacy of abemaciclib monotherapy in patients with advanced, "RB-positive" triple-negative breast cancer. For the purposes of the trial, we have defined RB positivity using an immunohistochemical assay – tumors in which greater than 50% of tumor cells stain positive for RB are deemed positive. In addition, to IHC, another important method for assessing RB function is through sequencing of DNA. Triple-negative tumors can often harbor either mutations or deletions in RB1, which confer a loss of RB functionality. Thus, we also wish to determine whether RB1 alterations at a genomic level might predict the response of triple-negative breast cancers to abemaciclib. In order to do this, we will access DNA sequencing data (where possible) on tumors from all patients who participate in the pre-screening process. For the majority of these patients, DNA sequencing will have already been performed using the Dana-Farber Cancer Institute's "Profile" targeted sequencing panel, as patients who sign consent for pre-screening in this study often also sign consent for other studies through which Profile testing is performed. Here, we propose to use this data to determine the impact of RB1 genomic alterations on response to abemaciclib, and also to explore correlations between RB1 genomic alterations and loss of RB staining on immunohistochemistry.

Moreover, recent studies have implicated other genomic alterations which may also confer resistance to abemaciclib (for example, in TP53, PIK3CA, PTEN, and other genes) [20]. As such, we will also interrogate the Profile data these and other genomic alterations, as an exploratory analysis to determine genomic correlates of response to abemaciclib.

Finally, recent data has suggested that CDK4/6 inhibitors can enhance anti-tumor immune responses not only through their effects on tumor cells, but also through direct effects on effector T lymphocytes (Deng, Cancer Discovery 2017; Schaer, Cell Reports 2018). As a result, we will also collect peripheral blood mononuclear cells from patients on study, with the intent of interrogating changes in T cell phenotype with abemaciclib therapy.

The gut microbiota has been recognized as a modulator of immune system development. Healthy individuals have microbial populations in their intestinal tract that vary markedly in composition. The diversity of intestinal microbiota represents a significant challenge to the host's immune defenses, which must balance immune tolerance of beneficial microbes with inflammatory responses against pathogens. Alterations in the gut microbiota and their resulting interactions with intestinal epithelieum and the host immune system are associated with many disease, including cancer. In addition, the identification of bacterial species associated with response could open new strategies to maximize the clinical benefit of cancer therapy through the modulation of gut microbiota.

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

3.1.1 Patients must have histologically or cytologically confirmed invasive breast cancer, which is recurrent, locally advanced, unresectable or metastatic.

3.1.2 Patients must have at least one lesion that is not within a previously radiated field and that is measurable per RECIST version 1.1. Bone lesions are not considered measurable.

3.1.3 Either the primary tumor and/or metastatic tumor must be triple-negative as defined below:

- Hormone receptor status: the invasive tumor must be ER- and PR-negative, or staining present in <1% by immunohistochemistry (IHC)
- HER2 status: the invasive tumor must be Human Epidermal Growth Factor Receptor 2 Negative (HER2-negative) by the ASCO CAP guidelines

In cases where both primary tumor and metastatic sample(s) have been tested for ER, PR, and HER2, then the triple-negative status of the tumor should be determined from the most recent sample available.

3.1.4 Either the primary tumor and/or the metastatic tumor must be RB positive as defined below:

- RB status: the invasive tumor must have greater than 50% of tumor cells staining positive for RB.

3.1.5 Prior Chemotherapy*:*

- Patients may have received 1-3 prior systemic therapies for metastatic disease (note: for patients who have first developed recurrent/metastatic disease within 12 months of completing any (neo)-adjuvant therapy for triple-negative breast cancer, the (neo)-adjuvant therapy is counted as a prior line of therapy).

- Patients must have been off treatment with myelosuppressive chemotherapy for at least 21 days or nonmyelosuppressive agents for 14 days before registration. Patients should also be adequately recovered (to baseline or grade 1) from acute toxicities of prior treatment except for residual alopecia and peripheral neuropathy.

3.1.6 Prior biologic therapy: Patients must have discontinued all biologic therapy at least 21 days before registration.

3.1.7 Prior radiation therapy: Patients may have received prior radiation therapy in either the metastatic or early-stage setting. Radiation therapy must be completed at least 7 days prior to study registration.

3.1.8 Patients on bisphosphonates or RANK-L inhibitors may continue receiving these therapies during study treatment. There is no washout period required between the last dose of these therapies and the start of abemaciclib.

3.1.9 The patient has an Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (ECOG scale provided as Appendix A)

3.1.10 Patients must have normal organ and marrow function as defined below:

- Absolute neutrophil count ≥1500/mm^3
- Platelets ≥100,000/mm^3
- Hemoglobin ≥8 g/dL

  Note: Patients may receive erythrocyte transfusions to achieve this hemoglobin level at the discretion of the investigator. Initial treatment must not begin earlier than the day the after the erythrocyte infusion

- Total Bilirubin ≤1.5x the upper limit of normal (ULN) or ≤ 2.0 x the upper limit of normal (ULN) in patients with documented Gilbert's syndrome.
- Serum creatinine ≤1.5 mg/dL OR calculated GFR ≥60mL/min
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤3 times the upper limit of normal. For patients with documented liver metastases, AST/ALT ≤ 5.0 times the upper limit of normal.

3.1.11 Female subjects of childbearing potential must have a negative serum pregnancy test at screening. Women of childbearing potential are defined as those who have not been surgically sterilized and have had a menstrual period in the past year

3.1.12 The patient must be ≥18 years old

3.1.13 Capable of understanding and complying with the protocol and has signed the informed consent document.

3.1.14 Able to swallow study drug.

3.1.15 Sexually active patients (male and female) must use medically acceptable methods of contraception during the course of the study and for 3 months after completion of study treatment. If a woman becomes pregnant or suspects she is pregnant while participating in this study, she should inform her treating physician immediately. While on the study and for 3 months after final drug administration, women may not breast-feed.

3.1.16 Confirmed availability of formalin-fixed, paraffin-embedded (FFPE) tumor tissue

3.1.17 Patients with tumor that is felt to be accessible to biopsy must be willing to provide tissue from a newly obtained core biopsy of a tumor lesion at baseline. Biopsies will be obtained up to 1 week (7 days) prior to initiation of treatment on Cycle 1, Day 1. Patients who undergo an attempted research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are not required to undergo a repeat biopsy in order to continue on protocol.

3.2 Exclusion Criteria

A patient will be excluded from the study if he or she meets **any** of the following criteria:

3.2.1 Received a prior CDK4/6 inhibitor.

3.2.2 Undergone major surgery within 14 days of the initial dose of study drug

3.2.3 Received another investigational agent (defined as any agent/device that has not received regulatory approval for any indication) within 14 days of the first dose of study drug for a nonmyelosupressive agent, or 21 days of the first dose of study drug for a myelosuppressive agent.

3.2.4 Has any severe concurrent disease, infection, or comorbid condition that renders the patient inappropriate for enrollment in the opinion of the investigator.

3.2.5 Has an active bacterial infection (requiring IV antibiotics at the time of initiating study treatment), fungal infection, or detectable viral infection. Patients with known HIV infection are excluded given the potential for interactions between antiretroviral agents and abemaciclib, and the potential for increased risk of life-threatening infection with therapy that is myelosuppressive. Patients with known Hepatitis B or Hepatitis C infection are excluded only if there is evidence of active infection (detectable Hepatitis B surface antigen, detectable Hepatitis C RNA)

3.2.6 Documented brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Participants with previously diagnosed brain metastases are eligible if they have completed treatment at least 7 days prior to trial registration, are neurologically stable, and have recovered from effects of radiotherapy or surgery.

- Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥2 weeks before the first study drug.
- Treatment for brain metastases may have included whole brain radiotherapy, radiosurgery, or a combination as was deemed appropriate by the treating physician.
- Patients who meet the above criteria and are clinically stable on anti-convulsant medication are eligible only if their anti-convulsant does not alter hepatic cytochrome P450 activity in a way that might interfere with metabolism of abemaciclib (see Appendix C).

3.2.7 Pregnant women are excluded from this study because of the potential for teratogenic effects.

3.2.8 Lactating women are excluding from the study.

3.2.9 Individuals with a history of a second malignancy are ineligible except for the following circumstances: individuals with a history of other malignancies are eligible if they have been disease-free for at least 5 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if they are diagnosed and have completed treatment within the past 5 years: cervical cancer in situ, and non-melanoma cancer of the skin. Patients with other cancers diagnosed within the past 5 years and felt to be at low risk of recurrence should be discussed with the principal investigator to determine eligibility.

3.2.10 Have received any live vaccination within 28 days of first dose of study drug.

3.2.11 Has a personal history of any of the following conditions: syncope of cardiovascular etiology, ventricular arrhythmia of pathological origin (including, but not limited to, ventricular tachycardia and ventricular fibrillation), or sudden cardiac arrest.

3.3 Inclusion of Women, Men, 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 PLAN

5.1 Treatment Regimen

Abemaciclib will be administered orally, twice daily on days 1 to 28 of a 28 day cycle as described in Table 5.1. Reported adverse events and potential risks 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.

TABLE 5.1 TREATMENT REGIMENS/DOSING SCHEDULE

Regimen	Period/Cycle	Dose Day
Abemaciclib	Treatment/28-day cycle	200 mg PO Q12H on Days 1-28
Abbreviations: PO=Orally	Q12H=every 12 (±2) hours.	

The investigator or his/her designee is responsible for the following:

- explaining the correct use of the drug and planned duration of each individual's treatment to the patient/site personnel/legal representative,
- verifying that instructions are followed properly,
- maintaining accurate records of study drug dispensing and collection

For assessing compliance with treatment, the participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle.

Patients will be instructed to contact the investigator as soon as possible if they have a complaint or problem with the study drug so that the situation can be assessed.

5.2 Pre-Treatment Criteria

On day 1 of cycle 1, blood will be drawn for complete blood count (CBC), liver function tests, urea, electrolytes, and creatinine. Results must be reviewed by the treating physician and meet the criteria below:

- Absolute neutrophil count ≥1500/mm^3
- Platelets ≥100,000/mm^3
- Hemoglobin ≥8 g/dL
- Total Bilirubin ≤1.5x the upper limit of normal (ULN)
- Serum creatinine ≤1.5 mg/dL OR calculated GFR ≥60mL/min
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤3 times the upper limit of normal. For patients with documented liver metastases, AST/ALT ≤ 5.0 times the upper limit of normal.

On day 1 of subsequent cycles, blood will be drawn for complete blood count (CBC), liver function tests, urea, electrolytes, and creatinine. Results must be reviewed by the treating

physician and meet the criteria below:

- Absolute neutrophil count ≥1000/mm^3
- Platelets ≥100,000/mm^3
- Total Bilirubin ≤1.5x the upper limit of normal (ULN)
- Serum creatinine ≤1.5 mg/dL OR calculated GFR ≥60mL/min
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤3 times the upper limit of normal. For patients with documented liver metastases, AST/ALT ≤ 5.0 times the upper limit of normal.

5.3 Agent Administration

5.3.1 Abemaciclib will be supplied as capsules for oral administration. The capsules should be stored at room temperature according to the range provided on the product label and not opened, crushed, or dissolved. Investigators should instruct patients to store the capsules in the original package and in a location inaccessible to children. Clinical study materials will be labeled according to country regulatory requirements.

5.3.2 Abemaciclib will be taken orally every 12 (± 2) hours on Days 1 through 28 of a 28-day cycle, for a total of 56 doses per cycle. Patients should not consume food beginning 1 hour before and ending 1 hour after taking abemaciclib. During all cycles, abemaciclib should be taken at approximately the same times each day. If a patient misses or vomits a dose, that dose should be omitted.

5.3.3 A patient may continue to receive study drug until she meets 1 or more of the specified reasons for discontinuation (as described in Section 5.5.)

5.4 General Concomitant Medication and Supportive Care Guidelines

With the exceptions provided in the sections below, no other chemotherapy, experimental medications, other anticancer therapy, immunotherapy, hormonal cancer therapy, surgery for cancer, or experimental medications will be permitted while patients are on study treatment. Radiation while on study treatment will be permitted after discussion with the PI.

The results from an in vitro human recombinant cytochrome P450 (CYP) phenotyping study indicate that oxidative metabolism of abemaciclib is primarily catalyzed by CYP3A4. However, the extent of oxidative metabolism responsible for the systemic clearance of abemaciclib in humans is unknown. Based on these in vitro findings, grapefruit juice as well as inducers (for example, phenytoin and carbamazepine) and strong inhibitors of CYP3A should be substituted or avoided if possible (Appendix C). If coadministration with a strong CYP3A inhibitor is unavoidable, reduce the abemaciclib dose to 100 mg twice daily or, in the case of ketoconazole, reduce the abemaciclib dose to 50 mg twice daily. In patients who have had a dose reduction to 100 mg twice daily due to adverse reactions, further reduce the abemaciclib dose to

50 mg twice daily. Avoid grapefruit or grapefruit juice. If a CYP3A inhibitor is discontinued, increase the abemaciclib dose (after 3-5 half-lives of the inhibitor) to the dose that was used before starting the inhibitor.

In vitro studies in primary cultures ofhuman hepatocytes indicate that abemaciclib might inhibit the metabolism of CYP2B6 substrate drugs in vivo. Based on this finding, sensitive substrates of CYP2B6 such as bupropion and efavirenz should be substituted or avoided if possible.

5.4.1 Supportive Care

Patients should receive full supportive care to maximize quality of life. Patients will receive supportive care based on the judgment of the treating physician. If it is unclear whether a therapy should be regarded as supportive care, the investigator should consult the principal investigator (PI).

5.4.2 Growth Factor Therapy

Growth factors may be administered in accordance with American Society of Clinical Oncology Guidelines [17]. Dosing of abemaciclib must be suspended if the administration of growth factors is required and must not be restarted within 48 hours of the last dose of growth factors having been administered. When restarted, the dose of abemaciclib must be reduced by 1 dose level if a dose reduction for the specific event necessitating the use of the growth factors has not already occurred.

5.4.3 Supportive Management for Diarrhea

At enrollment, patients should receive instructions on the management of diarrhea. In the event of diarrhea, supportive measures should be initiated as early as possible. These include the following:

- At the sign of loose stools, patients should initiate anti-diarrheal therapy (for example, loperamide) and notify the investigator/site for further instructions and appropriate follow-up.
- Participants should be encouraged to drink fluids (for example, 8 to 10 glasses of clear liquids per day)
- Site personnel should assess response within 24 hours
- If diarrhea does not resolve with anti-diarrheal therapy within 24 hours to either baseline or Grade 1, study drug should be held until diarrhea is resolved to baseline or grade 1.
- If diarrhea resolves to baseline or grade 1, participants should resume dosing as outlined in Section 6.3.2 and Table 6.1.

In severe cases of diarrhea, measuring neutrophil counts and body temperature and proactively managing diarrhea with antidiarrheal agents is recommended (page 16). In addition, patients with ≥ grade 3 diarrhea should be reviewed by a treating physician once per week until the diarrhea has resolved to ≤ grade 1.

If diarrhea is severe (requiring IV rehydration) and/or associated with fever or severe neutropenia, broad-spectrum antibacterial therapy, including coverage for enteric (gram-negative and
anaerobic) bacteria. If neutropenic fever persists for 5 days despite broad-spectrum antibacterial therapy, empiric antifungal therapy should be initiated. If diarrhea persists after antibiotic treatment, testing for *Clostridium difficile* is indicated as antibiotic therapy might precipitate *C. difficile* infection.

Patients with severe diarrhea or any grade of diarrhea associated with severe nausea or vomiting should be carefully monitored and given IV fluid (IV hydration) and electrolyte replacement. In these patients, electrolytes and renal function should be monitored regularly during the period of IV hydration.

5.5 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 until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the 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) and in the clinical trial management system, OnCore. Alternative care options will be discussed with the participant.

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

5.6 Duration of Follow Up

Participants should remain in long-term follow-up with disease status and overall survival status checked once every 6 months until death. There are no long-term follow-up visits required.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.7 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) and updated with the relevant Off Treatment/Off Study information in OnCore.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dose Adjustments and Delays

Table 6.1 Toxicity Dose Adjustments and Delays for Abemaciclib

Toxicity Type	Toxicity Profile and Severity	Dose	
Hematologic Toxicity Sections 6.2 and 5.4.2	Requiring administration of growth factors, regardless of severity (Growth factors use permitted according to ASCO Guidelines)	Dose **MUST** be suspended for at least 48 hours after the last dose of blood cell growth factors was administered and until toxicity resolves to at least Grade 2.	Dose **MUST** be reduced by 1 dose level unless already performed for incidence of toxicity that lead to the use of growth factor.
Hematologic Toxicity Section 6.2	Grade 3	Dose **MUST** be suspended until toxicity resolves to at least Grade 2.	Dose **MAY** be reduced by 1 dose level - investigator's discretion.
Hematologic Toxicity Section 6.2	Recurrent Grade 3	Dose **MUST** be suspended until toxicity resolves to at least Grade 2.	Dose **MUST** be reduced by 1 dose level.
Hematologic Toxicity Section 6.2	Grade 4	Dose **MUST** be suspended until toxicity resolves to at least Grade 2.	Dose **MUST** be reduced by 1 dose level.
Non-hematologic Toxicity (except diarrhea and increased AST/ALT) Section 6.3	Persistent or recurrent Grade 2 that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Dose **MUST** be suspended until toxicity resolves to either baseline or Grade 1.	Dose **MUST** be reduced by 1 dose level
Non-hematologic Toxicity (except diarrhea and increased AST/ALT) Section 6.3	Grade 3 or 4	Dose **MUST** be suspended until toxicity resolves to either baseline or	Dose **MUST** be reduced by 1 dose level.

		Grade 1.	
Diarrhea Sections 6.3.2 and 5.4.3	Grade 2	Dose **MUST** be suspended if toxicity does not resolve within 24 hours to ≤ Grade 1.	Dose reduction is not required.
Diarrhea Sections 6.3.2 and 5.4.3	Persistent or recurrent Grade 2 that does not resolve with maximal supportive measures within 24 hours to at least Grade 1	Dose **MUST** be suspended until toxicity resolves to at least Grade 1.	Dose **MUST** be reduced by 1 dose level - investigator's discretion.
Diarrhea Sections 6.3.2 and 5.4.3	Diarrhea recurs despite maximal supportive measures after resuming same dose level after initial Grade 2 diarrhea	Dose **MUST** be suspended until toxicity resolves to at least Grade 1.	Dose **MUST** be reduced by 1 dose level.
Diarrhea Sections 6.3.2 and 5.4.3	Requires hospitalization or Grade 3 or 4	Dose **MUST** be suspended until toxicity resolves to at least Grade 1.	Dose **MUST** be reduced by 1 dose level.
Hepatic Toxiciy (AST and/or ALT) Section 6.3.4	Persistent or recurrent Grade 2 (> 3.0-5.0 x ULN) OR Grade 3 (>5.0-20.0 x ULN) that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Dose **MUST** be suspended until toxicity resolves to baseline or Grade 1.	Dose **MUST** be reduced by 1 dose level.
Hepatic Toxiciy (AST and/or ALT) Section 6.3.4	Grade 3 (>5.0-20.0 x ULN) AND Total Bilirubin > 2.0 x ULN, in the absence of cholestasis.	Discontinue Abemaciclib.	N/A
Hepatic Toxiciy (AST and/or ALT) Section 6.3.4	Grade 4 (>20.0 x ULN)	Discontinue Abemaciclib.	N/A

Abbreviation: ASCO = American Society of Clinical Oncology.
Note: MAY = per the investigator's clinical judgment; SHOULD = not mandatory but highly recommended; MUST = mandatory.

6.1.1 Abemaciclib Dose Adjustments

If a patient who, in the judgment of the investigator, is receiving clinical benefit from study therapy requires further dose reduction than is outlined in Table 6.1, then the investigator must discuss with the PI prior to any further dose reduction. For patients requiring dose reduction(s), re-escalation to a prior dose level is permitted only after consultation with the PI.

A cycle is defined as the planned treatment interval of 28 days plus any subsequent delay prior to start of the next cycle. A delay in the start of a cycle due to holidays, weekends, bad weather, or other unforeseen circumstances will be permitted up to 7 days and not counted as a protocol deviation. No more than 56 doses of abemaciclib should be dispensed for each 28-day cycle. Dose omissions are allowed within a cycle. If a patient requires omission of more than 25% of doses during a cycle for tolerability, then treatment may continue if the investigator determines

the patient is receiving clinical benefit.

Table 6.1.1 Dose Adjustments of Abemaciclib

Dose Adjustment	Oral Dose	Frequency
0	200 mg	Every 12 hours
1	150 mg	Every 12 hours
2	100 mg	Every 12 hours
3	50 mg	Every 12 hours

6.2 Hematologic Toxicity

Hematologic toxicities including neutropenia, leukopenia, anemia, and thrombocytopenia have been observed in patients treated with abemaciclib, and causality has been established. Patients should be monitored closely for signs of infection, anemia, and bleeding. Dose adjustment may be considered for hematological toxicity ≥Grade 3. Hematological toxicities must resolve to baseline or at least Grade 2 prior to the start of each cycle. Blood cell growth factors are only to be used in a manner consistent with American Society of Clinical Oncology (ASCO) guidelines. Fever or infection in the presence of severe neutropenia should be managed promptly with broad-spectrum antibacterial therapy, including coverage for enteric (gram-negative and anaerobic) bacteria. If neutropenic fever persists for 5 days despite broad-spectrum antibacterial therapy, empiric antifungal therapy should be initiated. Anemia and thrombocytopenia should be treated supportively and if necessary with red cell or platelet transfusions.

Tables 6.1 and 6.1.1 provide further detail on abemaciclib dose modification for hematologic toxicity.

6.3 Non-hematologic Toxicity

6.3.1 Dose adjustment for other non-hematologic toxicity (other than diarrhea)

Before the start of each cycle, non-hematologic toxicity must be evaluated. All non-hematologic toxicities (except alopecia, diarrhea and fatigue) possibly related to abemaciclib must resolve to either baseline or at least Grade 1.

If a patient experiences persistent or recurrent Grade 2 non-hematologic toxicity (except diarrhea; refer to Section 6.3.2) possibly related to abemaciclib that does not resolve with maximal supportive measures within 7 days to either baseline or Grade 1, then dosing may be suspended (until the toxicity resolves to either baseline or at least Grade 1) and the dose of abemaciclib may be reduced as outlined in Table 6.1.1.

If a patient experiences ≥ Grade 3 non-hematologic toxicity possibly related to abemaciclib, then dosing must be suspended (until the toxicity resolves to either baseline or at least Grade 1) and the dose of abemaciclib must be reduced as outlined in Table 6.1.1.

6.3.2 Diarrhea

A patient experiencing diarrhea requiring hospitalization (irrespective of grade, that is, requiring intravenous [IV] rehydration) or severe diarrhea (Grade 3 or 4) must have study treatment suspended (until the toxicity resolves to either baseline or Grade 1) and should have the study drug dose reduced by 1 dose level as outlined in Table 6.1.

If a patient experiences persistent or recurrent diarrhea that does not resolve with maximal supportive measures within 24 hours to either baseline or at least Grade 1, then study treatment should be suspended (until the toxicity resolves to either baseline or at least Grade 1) and the dose of study drug may be reduced by 1 dose level as outlined in Table 6.1.1 at the discretion of the investigator. If the participant resumes treatment at the same dose level and diarrhea recurs despite maximal supportive measures, the dose of study drug must be reduced by 1 dose level as outlined in table 6.1.1.

6.3.3 General Guidance for Increases in Serum Creatinine and Assessment of Renal Insufficiency

Abemaciclib has been shown to increase serum creatinine due to inhibition of renal tubular transporters without affecting glomerular function (as measured by iohexol clearance). In clinical studies, increases in serum creatinine occurred within the first month of abemaciclib dosing, remained elevated but stable through the treatment period, were reversible upon treatment discontinuation, and were not accompanied by changes in markers of renal function, such as blood urea nitrogen (BUN), cystatin C, or calculated glomerular filtration rate based on cystatin C. Dose modifications are at the discretion of the treating provider, but none are stipulated by this protocol.

6.3.4 General Guidance for Hepatic Monitoring

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) elevation are considered expected with the use of abemaciclib. See Table 6.1 for dose modification guidance. Hepatic monitoring should depend upon the severity and persistence of the observed laboratory test abnormalities. If a study patient experiences elevated AST/ALT 5.0xULN and elevated total bilirubin 2.0xULN, or AST/ALT 8.0xULN, liver tests (including ALT, AST, TBL, direct bilirubin, gamma-glutamyl transferase (GGT), and creatinine phosphokinase (CPK), should be repeated within 3 to 5 days to confirm the abnormality and to determine if it is increasing or decreasing. If the abnormality persists or worsens, clinical and laboratory monitoring should be initiated by the investigator, based on the hepatic monitoring tests below:

Table 6.3.4: Hepatic Monitoring Tests for a Hepatic Treatment Emergent Abnormality.

Hepatic Hematology	**Haptoglobin**
Hemoglobin	
Hematocrit	**Hepatic Coagulation**
RBC	Prothrombin Time
WBC	Prothrombin Time, INR
Neutrophils, segmented and bands	
Lymphocytes	**Hepatic Serologies[a]**
Monocytes	Hepatitis A antibody, total
Eosinophils	Hepatitis A antibody, IgM
Basophils	Hepatitis B surface antigen
Platelets	Hepatitis B surface antibody
	Hepatitis B Core antibody
Hepatic Chemistry	Hepatitis C antibody
Total bilirubin	Hepatitis E antibody, IgG
Direct bilirubin	Hepatitis E antibody, IgM
Alkaline phosphatase	
ALT	**Anti-nuclear antibody**
AST	**Anti-actin antibody**
GGT	**Anti-smooth muscle antibody**
CPK	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; GGT = gamma-glutamyl transferase; Ig = immunoglobulin; INR = international normalized ratio; RBC = red blood cells; WBC = white blood cells.

a Reflex/confirmation dependent on regulatory requirements and/or testing availability

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Expected Toxicities

The most common AEs considered related to abemaciclib were headache (14%), nausea (11%), diarrhea (8%), and vomiting (8%).

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.

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

7.3 Expedited Adverse Event Reporting

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

7.3.2 DF/HCC Expedited Reporting Guidelines

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

7.4 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.

7.5 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.

7.6 [redacted]

SAEs will be reported to [redacted] on a MedWatch form as soon as possible and no later than 24 hours of the Investigator and/or Institution receiving notification of any serious adverse event experienced by a patient participating in the study and receiving study drug. Fax SAEs to the local safety representative using the following Fax number: 866-644-1697.

7.7 Routine Adverse Event Reporting

All Grade 2 or higher 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 AGENT INFORMATION

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

8.1 Abemaciclib

8.1.1 Description

Abemaciclib, (IUPAC: N-[5-[(4-ethylpiperazin-1-yl)methyl]pyridin-2-yl]-5-fluoro-4-(7-fluoro-2-methyl-3-propan-2-ylbenzimidazol-5-yl)pyrimidin-2-amine) , or LY2835219, is a potent and selective inhibitor of CDK4 and CDK6 with IC50 of 2 nM and 10 nM, respectively. It has a molecular weight of 602.7 and it chemical formula is C28H36F2N8O3S. The half-life is 17-38 hours.

8.1.2 Storage and Stability

Abemaciclib needs to be stored in the original package. Abemaciclib needs to be kept at room temperature (approximately 15-30 degrees Celsius wherever possible).

8.1.3 Handling

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

8.1.4 Availability

Abemaciclib will be provided for clinical trial use as 50mg of abemaciclib with excipients in hypromellose capsules as individual patient supply packaged in bottles. Storage conditions are described in the medication label.

8.1.5 Preparation

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

The investigator or responsible site personnel must instruct the participant or caregiver to take abemaciclib as per protocol. Abemaciclib will 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 CRF.

8.1.6 **Administration**

Abemaciclib is administered orally.

Compliance should be assessed by the investigator and/or study personnel at each participant visit and information provided by the participant and/or caregiver. Records of study medication used, dosages administered, and intervals between visits and the completion of the study will be captured in the Drug Accountability Form. This information must be captured in the source document at each participant visit.

8.1.7 **Ordering**

Abemaciclib will be provided to patients [redacted]

8.1.8 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

All drug supplies are to be used only for this protocol, and not for any other purpose.

8.1.9 **Destruction and Return**

The study drug supply will be destroyed according to the DFCI Institutional Pharmacy SOP.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Clinical specimens collected

The following clinical specimens will be collected from patients on this study:

a) Archival tumor tissue if available (paraffin-embedded)
b) Blood at baseline (for collection of plasma, germline DNA and ctDNA).
c) Blood at each time of restaging (for collecting ctDNA)
d) Blood at C1D15, C2D1, C3D1, and at progression (for plasma)
e) Tumor biopsy material obtained at time of study entry (if accessible disease) – both fresh frozen and formalin-fixed, paraffin-embedded

f) Tumor biopsy material obtained at time of C2D1 (if accessible disease) – both fresh frozen and formalin-fixed, paraffin-embedded
g) Blood at time of progression for collection of ctDNA
h) Stool at baseline, on treatment, toxicity, and EOT/PD

Sample	Type	Visit
Archival Tissue FFPE	Tumor	Screening
Fresh Tissue FFPE and Frozen	Tumor	Screening and Cycle 2 day 1
cfDNA	Blood	Screening, Restaging, and Progression
Plasma for isolation of exosomes	Blood	Screening, C1D15, C2D1, C3D1, and Progression
Germline DNA	Blood	Screening
PBMCs	Blood	Screening, C2D1, C3D1, and Progression
Stool	Stool	Screening, Between C2D1-C3D1, grade ≥ 2 diarrhea (optional), and EOT/PD

9.2 Archival Tissue Specimens

9.2.1 Collection of Archival specimen(s)

Archival tissue will be collected at screening to determine tumor RB status and potential eligibility for the trial. RB status will be determined using immunohistochemistry on formalin-fixed, paraffin embedded tissue.

The staining will be done using Rb antibody clone G3-245 using a protocol developed in the BWH clinical immunohistochemistry laboratory (CLIA conditions). A BWH pathologist (Dr Stuart Schnitt will review the stained slides and results will be reported to the study team. To be considered RB positive, the invasive tumor must have greater than 50% of tumor cells staining positive for RB. The expected turnaround time between receipt of tissue (slides or blocks) and delivery of the staining result is expected to be approximately 7-14 days.

We are requesting that 1 FFPE block or 15 unstained slides and 1 representative H&E stained slide containing tumor tissue be provided from each patient's definitive surgery or metastatic biopsy. The tissue requisition provided in (Appendix D) should be completed and submitted

with the tissue specimen and pathology report. If the archival specimen is from an outside hospital, please ensure the outside institutions pathology report is provided.

In addition to screening potentially eligible patients for RB-positivity by performing immunohistochemistry on archival tissue, we will also ask screened patients to consent for further studies on archival tissue when available. These studies will include (but will not be limited to) assessment of genomic and transcriptomic predictors of response to abemaciclib, as well as description of the correlations between RB-positivity by IHC and gene expression signatures in the tumor. In those patients that provide informed consent for this, we will perform both DNA and RNA extraction from the formalin-fixed, paraffin embedded archival tumor tissue available.

9.2.2 Handling of Archival specimen(s)

Once blocks/slides are received, RNA will be extracted from archival tumor tissue (**preferably the primary breast tumor**). This RNA will then be subjected to analysis of gene expression by Affymetrix microarray and/or using the Nanostring nCounter platform. Tumors will be classified according to published algorithms to determine their individual TNBC subtype. We expect that the majority (but not all) of the RB-positive tumors will fall into the "Luminal Androgen Receptor" or "Mesenchymal Stem Like" subtypes, and as an exploratory analysis will determine if tumors within this subtype have a higher rate of response to abemaciclib.

Additionally, in patients who consent, RNA extracted will be used to quantify the expression of several genes, including (but not limited to) those used to classify tumors into PAM50 subtypes[18], those identifying specific TNBC subtypes [19][8], and those that contribute to a signature of RB proficiency or deficiency. This will allow us to depict the landscape of gene expression for all tumors in this study, and also to determine in an exploratory fashion the correlation (or lack thereof) between RB positivity by IHC and "Rb-proficiency" by gene expression. DNA extracted will be stored with a view to performing whole exome sequencing. This will allow a depiction of the landscape of genomic alterations of all patients screened for the study, and specifically allow for an exploratory comparison of mutational profiles in tumors that do and do no stain positively for RB.

9.2.3 Site Performing Archival Tissue Studies

9.3 Circulating tumor DNA (ctDNA)

Blood will be collected at baseline, restaging visits and at time of progression for evaluation of circulating tumor DNA (ctDNA). The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.3.1 Collection of ctDNA 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 ctDNA. 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.

9.3.2 Handling and shipping of ctDNA specimens

One 10 ml Streck tube will be collected and processed at baseline, restaging visits and at time of progression for evaluation of ctDNA. 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:

[redacted]

Email the blood bank [redacted] 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 REFRIGERATE TUBES as this could result in ctDNA 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., Study Biopsy Specimens, Streck Tubes) 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.

Blood in these tubes will be processed for collection of plasma (for ctDNA) See

Appendix B for additional instructions on use of Streck tubes.

9.4 Collection of Plasma Samples for Exploratory Biomarker Evaluations

EDTA-anticoagulated plasma samples will be collected and analysis may be performed on biomarkers that may play a role in the LY2835219 mechanism of action. The evaluation of these samples may involve analysis of DNA, RNA, and proteins (including any of these components derived from exosomes) to investigate their association with observed clinical outcomes to study drug.

9.4.1 Handling and shipping of plasma specimens:

One 10 mL purple top EDTA tube should be collected at C1D1, C1D15, C2D1, C3D1 and at time of progression. These samples should be sent to:

Once collected, the tube should be centrifuged, and the plasma separated into 2 aliquots and frozen in the Clinical Research Laboratory.

9.5 Germline DNA

The objective for collecting germline DNA is to perform germline exome sequencing as a control for the paired tumor biopsies.

9.5.1 Handling and shipping of plasma specimens:
One 10 ml Streck tube will be collected and processed at baseline for germline DNA. 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:

Email the blood bank [redacted] 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 REFRIGERATE TUBES as this could result in ctDNA 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., Study Biopsy Specimens, Streck Tubes) 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.

Blood in these tubes will be processed for collection of the buffy coat for germline DNA. See Appendix B for additional instructions on use of Streck tubes.

9.6 PBMCs

As PBMC collections were added after the study began enrolling, collections will begin with the first patient enrolled after the activation of DFCI Amendment #6.

9.6.1 Handling and shipping of PBMC specimens:

Five 10 ml EDTA tubes will be collected and processed at baseline, C2D1, C3D1, and progression for PBMCs. Fill the tubes completely and deliver to the Center for Immuno-Oncology Lab within 3-4 hours of draw at the following address:

The CIO lab staff will send reminders each week; however, please notify the lab of any planned draws so that they can staff appropriately.

9.6.2 Potential Testing: Flow Cytometry

PBMCs will be generated as described in the appendix, and used to assess immune cell populations.

Surface staining with a panel of antibodies and flow cytometry on PBMCs will then be performed as described in Appendices. The following antibodies will be used on all specimens: (core set) CD8, PD-1, PD-L1, PD-L2.

A selection of the following antibodies may also be used, and additional antibodies may be used

as well, as deemed appropriate and informative based on the state of the immune profiling literature at the time of correlative science performance: CD4, FOXP3, CD127.

9.6.3 Site Performing Correlatives:

DFCI CIO

9.7 Analysis of tissue biopsy specimens obtained at baseline and at C2D1

9.7.1 Guidelines for Tissue Acquisition and Biopsies of Metastatic Lesions

9.7.1.1 Collection of Specimen(s)

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 the baseline biopsy, ideally five core biopsies will be obtained and processed as follows:

- First core will be placed in 10% neutral buffered formalin and then made into a formalin fixed paraffin embedded (FFPE) block.
- Second core will be frozen in OCT
- Third core will be placed in 10% neutral buffered formalin and then made into an FFPE block.**
- Fourth core will be frozen in OCT**
- Fifth core will be placed in DMEM

**If a biopsy yields less than five cores, the PI may opt to have the 3rd or 4th core placed in DMEM to send to the lab of Dr. Peter Sicinski. This can be discussed with Dr. Goel in advance of the procedure.

For the C2 biopsy, ideally five core biopsies will be obtained and processed as follows:

- First core will be placed in 10% neutral buffered formalin and then made into a formalin fixed paraffin embedded (FFPE) block.
- Second core will be frozen in OCT
- Third core will be placed in 10% neutral buffered formalin and then made into an FFPE block.
- Fourth core will be frozen in OCT

- Fifth core will be frozen in OCT

Guidelines for biopsy from various metastatic sites can be found below.

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 3 x 4-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 11-13 gauge needle.

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

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

9.7.1.2 Site(s) Performing Correlative Study

Dana-Farber Cancer Institute

9.7.1.3 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.

9.7.1.4 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. 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.

9.7.1.5 Shipping of tissue specimens

Specimens in OCT and formalin will be delivered to the Clinical Trials Core Laboratory for storage:

[redacted]

Email the blood bank [redacted] and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

The core in DMEM will be delivered to the lab of Dr. Peter Sicinski immediately following collection for processing:

[redacted]

Email Anne Fassl in the lab [redacted] at least 24 hours before of the procedure. If the biopsy does not yield enough tissue to collect the 5th core, notify the lab that the procedure was performed, but did not yield a core, immediately following the biopsy. The lab will provide 10mL of DMEM in 15mL conical tubes for the collection of specimens, to be stored in a central location.

Coded laboratory specimens will be stored in the Clinical Trial Core Laboratory. These specimens will become the property of DFCI. 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 will be performed by Dr. Shom Goel in the Department of Cancer Biology at DFCI or relevant core facilities at DFCI.

9.7.1.6 Analysis of tissue specimens obtained from biopsies at baseline and C2D1

<u>Analysis of baseline biopsy</u>

Tumor tissue obtained from the baseline biopsy will be used to examine candidate markers of sensitivity or resistance to abemaciclib therapy, based on available preclinical and clinical data.

a) DNA: extracted DNA will be subject to whole exome sequencing as an exploratory analysis. Specifically, we will determine copy number for cyclin D1 and cyclin E1/2, and also mutational data for TP53 (approximately 60-80% of TNBCs contain a TP53 mutation). We will determine whether the rate of response is lower in tumors cyclin E amplification, or tumors with TP53 mutations. Exploratory analyses will also be performed to determine other novel predictors of response to abemaciclib.
b) RNA: extracted RNA will be used to determine expression levels of genes including but not limited to, cyclin D1, cyclin E1, cyclin E2, CDKN2A, CDKN1A, CDKN1B, CDK2, TSC2, and PDK1. In each case, will provide descriptive statistics comparing response rates between tumors with higher or lower levels of expression of these genes.
c) Protein: Baseline core biopsies will be stained for androgen receptor (AR) and RB. We expect that most, but not all, tumors will be AR-positive. We will determine response rates in patients with AR-positive vs AR-negative tumors. We will also document the concordance rate between RB positivity in archival and metastatic samples.
d) Fresh tissue: when at least 3-5 cores are obtained from baseline biopsies, 1 will be delivered fresh to the laboratory of Dr Peter Sicinski (DFCI). The Sicinski lab team will dissociate this tissue to isolate cancer cells and test novel methods to enhance the efficacy of CDK4/6 inhibition in TNBC cells.

A portion of the baseline biopsy will have RNA extracted. This RNA will then be subjected to analysis of gene expression by Affymetrix microarray and/or using the Nanostring nCounter platform. Tumors will be classified according to published algorithms to determine their individual TNBC subtype. We expect that the majority (but not all) of the RB-positive tumors will fall into the "Luminal Androgen Receptor" or "Mesenchymal Stem Like" subtypes, and as an exploratory analysis will determine if tumors within this subtype have a higher rate of response to abemaciclib. Additionally, in patients who consent, RNA extracted will be used to quantify the expression of several genes, including (but not limited to) those used to classify tumors into PAM50 subtypes[18], those identifying specific TNBC subtypes [19][8], and those that contribute to a signature of RB proficiency or deficiency. This will allow us to depict the landscape of gene expression for all tumors in this study, and also to determine in an exploratory fashion the correlation (or lack thereof) between RB positivity by IHC and "Rb-proficiency" by gene expression. The Nanostring assay will be performed at the BWH Center for Advanced Molecular Diagnostis (CAMD). Some portion of de-identified patient clinical information will be shared with Nanostring Technologies, with patient permission, in exchange for the kits needed to perform this assay.

<u>Analysis of paired baseline and C2D1 biopsies</u>

a) <u>Phospho-RB:</u> FFPE tissue from baseline and C2D1 biopsies will be stained for p-RB (ser780) using protocols established at the Brigham and Women's Hospital. Sections will be scored manually and the percentage of positive tumor cells will be recorded – a minimum of 200 cells will be counted. A tumor H score will be calculated. We will determine whether (i) baseline RB phosphorylation level predicts response to abemaciclib and (ii) whether suppression of RB phosphorylation after commencement of therapy is associated with an increased response rate.

b) FFPE tissue from baseline and C2D1 biopsies will be stained for proteins involved in the regulation of cell proliferation in the lab of Kornelia Polyak, MD, PhD at Dana-Farber Cancer Institute.

9.8 Research Stool Collection

9.8.1 Collection

All stool samples will be collected by each patient at home using a home-based, self-collection and mail method for stool that has been proven to provide nearly equivalent metagenomic and metatranscriptomic data to state-of-the-art fresh-frozen sample-collection protocol.

Patient questionnaires are included in the kits. The questionnaires should be completed at the time of stool collection and mailed back along with the sample. Stool samples and questionnaires that are not collected at the protocol-specified collection time points will not be protocol violations.

Given that this procedure was added to the study mid-way through accrual, only new participants will be approached to participant in this correlative study. If a patient doesn't complete a baseline collection, the series may not continue as this collection is essential to analysis.

9.8.2 Handling and shipping of stool specimens

All kits will be provided to the patients at a clinic visit or will be mailed to their home address. Patients will also be provided with a mailer in which to return the sample. All samples will be shipped by the patient at ambient temperature to the Breast Tumor Immunology Lab (BTIL, Elizabeth Mittendorf, MD, PhD) where they will be processed and stored until batch shipment for analysis.

9.8.3 Analysis

We will quantify microbiome features from amplicon, metagenome, metatranscriptome using established pipelines to identify strain-level taxonomic, functional gene, transcriptional, and microbially-mediated metabolite profiles associated with patients with Rb+ TNBC treated with abemaciclib.

9.8.4 Sites performing correlative analysis

Dana-Farber Breast Tumor Immunology Lab (BTIL, Elizabeth Mittendorf, MD, PhD)
Microbiome Dx

10. STUDY CALENDAR

Baseline evaluations are to be completed within 28 days prior to registration unless otherwise specified. Laboratory assessments, including pregnancy test, are to be completed within 14 days of registration. 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 $\pm$ 3 days of the protocol-specified date, unless otherwise noted. Tumor assessments must be completed within $\pm$ 7 days of the protocol-specified date.

	Screening	Cycle 1		Cycle 2		Cycle 3	Cycle 4+		30 Day FUV[k]	Follow -Up
		D1	D15	D1	D28	D1	D1	D28		
Informed consent	X									
Demographics	X									
Medical history, including past lines of therapy	X									
Physical exam	X	X	X	X		X	X		X	
Vital signs[a]	X	X	X	X		X	X		X	
Height	X									
Weight	X	X		X		X	X		X	
Performance status	X	X		X		X	X		X	
Concurrent Meds	X	X	X	X		X	X		X	
AE Evaluation	X	X	X	X		X	X		X	
CBC w/diff	X	X	X	X		X	X		X	
Serum chemistry[b]	X	X	X	X		X	X		X	
Serum cystatin C[c]	X	X	X	X		X	X		X	
B-HCG	X[d]									
Blood for ctDNA[e]		X				X			X	
Blood for genomic sequencing control		X								
Blood for plasma exosome analysis[f]		X	X	X		X			X	
Blood for germline DNA[g]		X								
Blood for PBMCs	X			X		X			X	
Archival tumor tissue	X									
Research stool collection and questionnaire[m]	X			X			X		X	
Research Biopsy	X			X						
EKG[h]	X									
Tumor assessments[i]	X				X			X	X[j,k]	
Abemaciclib		X----------						X		
Survival Follow-up										X

Abemaciclib: 200mg po bid for 28 day cycles.

a. Vital signs to include: diastolic and systolic blood pressure, heart rate and temperature
b. Chemistry to include: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
c. Cystatin C should be performed each time serum chemistries are performed, as abemaciclib is known to compete for tubular secretion of creatinine, causing an increase in serum creatinine which does not reflect changes in renal function. Cystatin C allows for more accurate assessment of renal function in this context.
d. Serum pregnancy test (women of childbearing potential as defined in the eligibility section)
e. ctDNA to be drawn at C1D1, each restaging vist and at time of progression/30 Day FUV (Streck)
f. Plasma to be drawn at C1D1, C1D15, C2D1, C3D1 and at time of progression/30 Day FUV (EDTA)

g. Blood drawn at C1D1 for collection of germline DNA (Streck) h. EKGs to be done at Screening and if clinically indicated i. Tumor assessments will be performed every 2 cycles (8 weeks) for the first 6 cycles (6 months) and then every 3 cycles (12 weeks) going forward. j. Tumor assessments should be obtained at 30 day FUV if not done within the previous 8 weeks k. Follow-up survival (disease status and overall status) follow-up evaluation – a separate clinic visit is not required, this can be done over the phone every six months. l. 30 Day FUV to be completed within 30 days of last dose of abemaciclib m. Research stool collection to occur at baseline, between C2D1 and C3D1, at the time of grade ≥ 2 diarrhea, and at the end of treatment or progression, whichever occurs first.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

A baseline tumor evaluation must be performed within 4 weeks before patient begins study treatment. Restaging scans will be performed every 8 weeks.

Response and progression will be evaluated in this study using the international criteria proposed by the New Response Evaluation Criteria in Solid Tumors (RECIST): Revised RECIST Guideline (version 1.1; Eisenhauer et al. 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

Tumor lesions/lymph nodes will be categorized at baseline as measurable or non-measurable. Measurable disease is defined by the presence of at least 1 measurable lesion.

11.1.2.1 Measurable

Tumor lesions: Measured in at least 1 dimension (longest diameter in the plane of

measurement is to be recorded) with a minimum size of:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (slice thickness ≤5 mm)
- 10 mm caliper measurement by clinical exam (non-measurable lesions if cannot be accurately measured with calipers)
- 20 mm by chest X-ray.

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 ≤5 mm).

11.1.2.2 Nonmeasurable

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitis involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measureable by reproducible imaging techniques.

11.1.2.3 Special Considerations for Lesion Measurability

Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI, can be considered measurable lesions if the soft tissue component meets the definition of measurability.
- Blastic bone lesions are non-measurable.

Cystic Lesions:

- Simple cysts should not be considered as malignant lesions (neither measurable nor nonmeasurable)
- Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability. If non-cystic lesions are presented in the same patients, these are preferred for selection as target lesions.

Lesions with Prior Local Treatment:

- Tumor lesions situated at a previously irradiated area, or in an area subjected to other loco-regional therapy, are non-measurable unless there has been demonstrated progression in the lesion.

11.1.3 Target lesions

When more than 1 measurable lesion is present at baseline, all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded an measured at baseline. Non-nodal target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and can be reproduced in repeated measurements. Measurable lymph nodes are target lesions if they meet the criteria of a short axis of ≥15 mm by CT scan. All measurements are to be recorded in the case record form (CRF) in millimeters (or decimal fractions of centimeters [cm]).

11.1.4 Non-target lesions.

All other lesions (or sites of disease) are identified as non-target lesions (chosen based on their representativeness of involved organs and the ability to be reproduced in repeated measurements) and should be recorded at baseline. Measurement of these lesions are not required but should be followed as 'present,' 'absent,' or in rare cases 'unequivocal progression.' In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the CRF (for example, multiple liver metastases recorded as 1 liver lesion).

Lymph nodes with short axis ≥10 mm but <15 mm should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and are not recorded or followed.

11.1.5 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.

An adequate volume of a suitable contrast agent should be given so that the metastases are demonstrated to best effect and a consistent method is used on subsequent examinations for any given patient. If prior to enrollment it is known a patient is not able to undergo CT scans with IV contrast due to allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (with or without IV contrast) should be used to evaluate the patient at baseline and follow-up should be guided by the tumor type under investigation and the anatomic location of the disease.

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. When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may be reviewed at the end of the study.

Chest x-ray. Chest CT is preferred over chest X-ray when progression is an important endpoint. Lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

Conventional CT and MRI. CT scan is the best currently available and reproducible method to measure lesions selected for response assessment. Measurability of lesions on CT scan is based on the
assumption that CT slice thickness is ≤5 mm. When CT scan have slice thickness >5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (for example, for body scans). If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Pet Scan (FDG-PET, PET CT): PET is not recommended for lesion assessment. If a new lesion is found by PET, another assessment must be done by CT, unless the PET CT is of diagnostic quality. If CT is done to confirm the results of the earlier PET scan, the date of progression must be reported as the earlier date of the PET scan.

Ultrasound. Ultrasound should not be used to measure lesion size. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date 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.

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. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

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).

When effusions are known to be a potential adverse effect of treatment (for example, with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease (SD) in order to differentiate between response (or SD) and progressive disease (PD).

Bone Scan: If lesions measured by bone scan are reported at baseline, it is necessary to repeat the bone scan when trying to identify a complete response (CR) or partial response (PR) in target disease or when progression in bone is suspected.

11.1.6 Response Criteria

11.1.6.1 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. 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.

Not Evaluable: When an incomplete radiologic assessment of target lesions is performed or there is a change in the method of measurement from baseline that impacts the ability to make a reliable evaluation of response.

11.1.6.2 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).

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.

*Not Evaluable: Wh*en a change in method of measurement from baseline occurs and impacts the ability to make a reliable evaluation of response.

11.1.6.3 Evaluation of New Lesions

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

11.1.6.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the earliest of objective progression or start of new anticancer therapy, taking into account any requirement for confirmation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. The Best Overall Response will be calculated via an algorithm using the assessment responses provided by the investigator over the course of the trial.

11.1.6.5 Time Point Response

It is assumed that at each protocol-specified time point, a response assessment occurs. (When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point.) Table 11.1.4.5 provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

Table 11.1.4.5 Time Point Response: Patients with Target (+/- Nontarget) 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	
Not all evaluated	Non-PD	No	SD	

Abbreviations: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; NE = inevaluable.

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

11.1.7 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that 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.

11.1.8 Response Review

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

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0.

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) 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 Office of Data Quality in accordance with DF/HCC SOPs.

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.

12.3 Collaborative Research and Future Use of Data and Specimens

Tissue, blood, bodily fluids, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one

of more publicly-accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a single-arm, two-stage phase 2 study to evaluate the response to abemaciclib monotherapy in patients with Rb-positive, triple negative metastatic breast cancer.

13.1.1 Primary objective and endpoint

To evaluate the efficacy of abemaciclib, as defined by objective response rate (ORR) in patients with Rb-positive triple-negative metastatic breast cancer (ORR as confirmed Complete Response (CR) or Partial Response (PR) per Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1).

13.1.2 Secondary efficacy endpoints

1) Progression-Free Survival (PFS)
2) Overall Survival (OS).
3) Disease control rate (DCR)
4) Clinical benefit rate (CBR)

13.2 Sample Size, Accrual Rate and Study Duration

This is a single-arm, two-stage phase 2 study in order to determine the efficacy of abemaciclib monotherapy in patients with metastatic RB-positive TNBC. There is no data for response to abemaciclib in patients with TNBC who are RB-positive. A response rate of 20% would be of clinical interest. A true response rate of 5% would not be of clinical interest. We have used a Simon optimal two-stage model with a one-sided type I error of 10% and type II error of 0.1 (90% power) to detect the difference between null (5%) and alternative (20%) objective response rates. The first stage of the study includes 12 patients with metastatic triple-negative breast cancer which is RB-positive. As of June 2018, it was discovered that a participant without measurable disease was registered and treated on this trial. As such, this participant is considered unevaluable in relation to the primary endpoint. Therefore, we plan to enroll a total of 13 participants to the first stage, with the expectation that 12 will be evaluable for response. The statistical plan will otherwise remain unchanged. If in the first stage there is at least one

response, accrual will continue to the second stage where an additional 25 patients will be enrolled. If there are at least 4 responses among the 37 evaluable patients, the regimen would be considered to be of clinical benefit worthy of further study (exact alpha=0.09). If the true the response rate is 5%, the probability that the study would discontinue after stage I is 54%. The total accrual to the study will be 38 given the additional patient added for evaluation in the first stage.

13.3 Analysis of Primary Endpoints

Best overall response will be assessed among patients who received at least one dose of protocol therapy using RECIST 1.1 criteria. The objective response rate (CR+PR) will be reported with 95% confidence interval adjusting for two-stage design.

13.4 Analysis of Secondary Endpoints

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 randomization (or registration) to the earlier of progression (per RECIST 1.1) or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

PFS and OS will be described using the methods of Kaplan-Meier, and they will be presented with 95% confidence intervals.

Disease control rate: Disease control is defined as CR, PR, or SD ≥ 16weeks per RECIST 1.1. Disease control rate will be reported with a 95% exact confidence interval.

Clinical benefit rate: is defined as CR, PR, or SD ≥ 24weeks per RECIST 1.1. Clinical benefit rate will be reported with a 95% exact confidence interval.

Phospho-Rb reduction will be evaluated as a dichotomous endpoint from paired tumor assessment. It is anticipated that 60-70% of patients will have reduction in phospho-Rb after one cycle of protocol treatment. Due to the low anticipated response rate, disease control will be used as the primary evaluation of the marker in order to maintain power to detect an association with phospho-Rb reduction. Based on data from the Monarch-1 study in ER+ breast cancer (Dickler et al, ASCO 2016) a 34% disease control rate in the total study population would be anticipated if abemaciclib monotherapy is observed to have activity (lower than that observed in ER+ disease).

Assuming that 33 pairs of biopsies were evaluable for phosphor-Rb (10% failure rate), we will have more than 85% power to detect a difference in the disease control rate if the rates are 53.3% among patients with phospho-Rb reduction and 5% among patients without phospho-Rb reduction. The following table gives the power of detecting the difference in disease control among patients with and without phospho-Rb reduction,

assuming the overall rate is 34% and with incidences of 60% and 70% phospho-Rb reduction

# of paired biopsies evaluable for phosphor-Rb	Expected tumor with phospho-Rb reduction	DCR among pts with phospho-Rb reduction	DCR among pts without phospho-Rb reduction	Power
29	60%	53.3%	5%	81%
		50%	10%	56%
	70%	47.7%	2%	73%
		46.4%	5%	58%
33	60%	53.3%	5%	85%
		50%	10%	63%
	70%	47.7%	2%	76%
		46.4%	5%	60%

.
Phospho-Rb reduction will be evaluated using Fisher Exact test with two-sided alpha = 0.05. Exploratory analyses will additionally consider the other efficacy endpoints and quantitative measures of phospho-Rb reduction using logistic regression models. With a 20% probability of objective response overall, there will be 89% power to detect an improvement to 50% probability of response with a 1 standardized-unit increase in phosph-RB reduction (OR = 4.0). Power calculations were performed in East v6.3 (Cytel Inc.).

13.5 Analysis of Exploratory Endpoints

Descriptive statistics will be used to characterize any types of biomarkers discovered in correlative work, T-cell phenotypes via serial PBMCs blood draws (prior to receiving therapy, while on therapy, and at the time of progression), any changes in the serial microbiome collection, and its correlation with participant outcomes.

13.6 Reporting and Exclusions

13.6.1 Evaluation of toxicity

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

13.6.2 Evaluation of the Primary Efficacy Endpoint

All Participants who receive at least one dose of either study drug will be evaluable for

efficacy endpoints. Participants who never start protocol therapy will be considered inevaluable and will be replaced. Biomarker analyses will be conducted in all Participants with evaluable biospecimen.

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: USE OF STRECK TUBES

Cell-Free DNA BCT®

Streck

INSTRUCTIONS FOR USE

INTENDED USE

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

SUMMARY AND PRINCIPLES

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

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

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

REAGENTS

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

PRECAUTIONS

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

STORAGE AND STABILITY

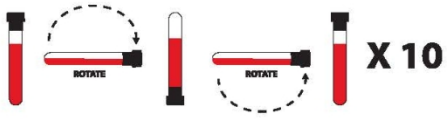
1. When stored at 18-30°C, unused Cell-Free DNA BCT is stable through expiration date.
2. Do not freeze unfilled Cell-Free DNA BCT. Proper insulation may be required for shipment during extreme temperature conditions.
3. Blood samples collected in Cell-Free DNA BCT for cf-DNA analysis are stable for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

INDICATIONS OF PRODUCT DETERIORATION

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

INSTRUCTIONS FOR USE

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



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

CELL-FREE PLASMA DNA AND CELLULAR GENOMIC DNA EXTRACTION

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

Note:

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

LIMITATIONS

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

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ORDERING INFORMATION

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

GLOSSARY OF HARMONIZED SYMBOLS

EC REP Authorized Representative in the European Community	LOT Batch Code	Biological Risk	REF Catalog Number	Use By
IVD In Vitro Diagnostic Medical Device	Manufacturer	Consult Instructions For Use	Temperature Limitation	Do Not Re-use
Glossary of symbols may contain symbols not used in the labeling of this product.				

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

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2014-04

APPENDIX C: INDUCERS AND STRONG INHIBITORS OF CYP3A4 OR SUBSTRATES OF CYPS WITH NARROW THERAPEUTIC RANGE

Inducers of CYP3A4

Carbamazepine
Dexamethasone
Phenobarbital/phenobarbitone
Phenytoin
Rifapentine
Rifampin
Rifabutin
St. John's wort

Strong Inhibitors of CYP3A4

Nefazodone
All HIV protease inhibitors
Clarithromycin
Itraconazole
Ketoconazole

Cytochrome P450 Substrates with Narrow Therapeutic Range

CYP1A2
Theophylline
Tizanidine

CYP2C8
Paclitaxel

CYP2C9
Warfarin
Phenytoin

CYP2D6
Thioridazine
Pimozide

APPENDIX D GENERATION OF PBMCS

1. Pour blood from green-cap tubes (heparin treated tubes) into two 50 ml conical tubes (Corning, 430290).
2. Spin tubes at 1500 rpm for 10 min (Sorvall Legend XTR centrifuge).
3. Aspirate 2 ml plasma/tube and aliquot into 4 tubes mircocentrifuge tubes (Fisherbrand, 05-408-138)
4. Spin plasma at 3000 RPM for 5 minutes (Sorvall Legend Micro 21R centrifuge)
5. Aspirate plasma into Cryogenic tubes 2 ml plasma/ tube (Corning, 430488).
6. Dilute blood 1:1 with PBS. (Blood amount should not exceed 25 ml per tube.)
7. Take 2 new 50 ml conical tubes and add 12 ml ficoll-paque (Cat# 17144003; GE Healthcare) per tube.
8. Slowly and gently layer the diluted blood on top of the ficoll-paque of the tube with a maximum volume of 35 ml.
9. Centrifuge the tube at 1900 rpm for 20 min at room temperature with slow acceleration (#7) and deceleration (#7) (Sorvall Legend XTR centrifuge).
10. Remove the PBMC layer from between the upper layer (diluted plasma) and middle layer (ficoll-paque) and transfer into a 50 ml conical tube. The lower layer is composed of red blood cells.
11. Completely fill conical tube containing isolated PBMC with PBS, mixing well.
12. Count viable cells by mixing 10 µl Trypan Blue with 10µl PBMC/PBS dilution in a microcentrifuge tube. Load 10 µl of mixture onto Countess Cell Counting Chamber Slide (Invitrogen, C10283) and read with Countess Automated Cell Counter (Invitrogen).
13. Centrifuge the tubes containing PBMC/PBS mixture at 1500 rpm for 5 min at room temperature (Sorvall Legend XTR centrifuge).
14. Remove PBS, and resuspend PBMC pellet in appropriate amount of freezing solution so that there are approx 5×10^6 cells/cryo vial in 300-500 µl of Fetal Bovine Serum (heat inactivated) plus 15% DMSO.
15. Put vials in CoolCell container (Biocision Inc.) and transfer to -80C freezer overnight.
16. Transfer cells to liquid nitrogen tank