DF/HCC Protocol #: 23-355

TITLE: The TRADE study: A Phase 2 Trial to Assess the ToleRability of Abemaciclib Dose Escalation in Patients with Early-Stage HR-positive and HER2-negative Breast Cancer

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Industry-supplied agent: Abemaciclib (LY2835219, Verzenio™) *supplied by Eli Lilly and Company*
Other Agents: Aromatase inhibitor (letrozole, anastrozole, or exemestane), tamoxifen, LHRH modulators, *commercially supplied*

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

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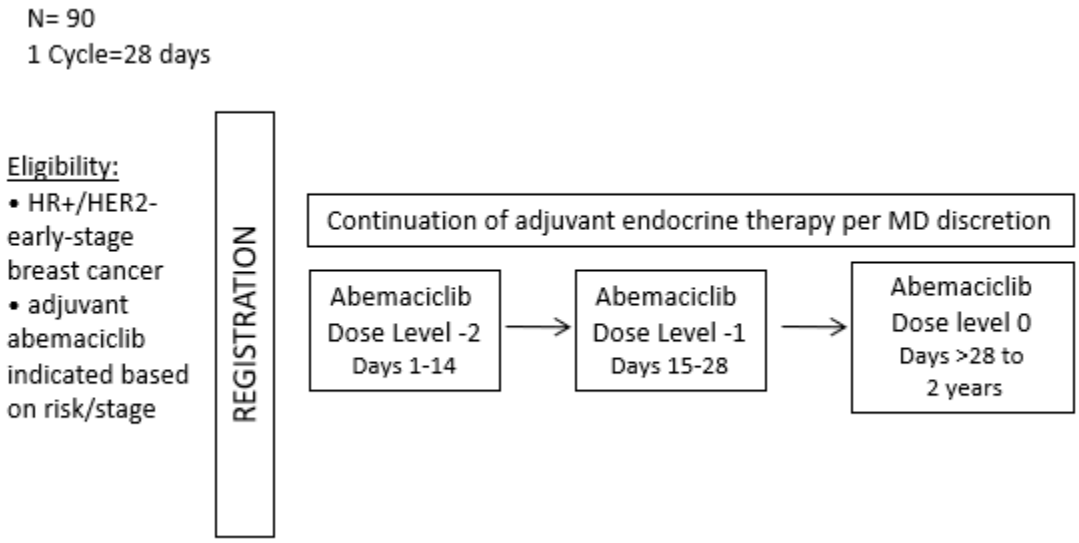
SCHEMA


N= 90
1 Cycle=28 days
Eligibility:
• HR+/HER2- early-stage breast cancer
• adjuvant abemaciclib indicated based on risk/stage
REGISTRATION
Continuation of adjuvant endocrine therapy per MD discretion
Abemaciclib Dose Level -2 Days 1-14
Abemaciclib Dose Level -1 Days 15-28
Abemaciclib Dose level 0 Days >28 to 2 years

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1. OBJECTIVES

1.1 Study Design

TRADE is a prospective, single-arm, open label, phase 2 study designed to evaluate a short-course dose-escalation strategy for adjuvant abemaciclib for the improvement of drug tolerability. The primary endpoint is a composite rate of abemaciclib discontinuation, dose modification, and/or inability to reach or maintain the full dose at 12 weeks.

Eligible patients have early-stage, high-risk, node-positive HR+/HER2- breast cancer. Patients who have completed primary surgical, radiotherapy, and chemotherapy treatments (if indicated), and have a medical indication for adjuvant abemaciclib per standard clinical practice, will be potentially eligible. Participants may be either pre- or post-menopausal.

Participants will receive an initial dose-escalated modified schedule of abemaciclib in combination with ongoing provider-choice endocrine treatment per institutional standard of care. All patients will start abemaciclib at the -2 dose level (50 mg BID) for the first 2 weeks of therapy. Patients will proceed to the next dose level only if they do not experience adverse events qualifying for dose reduction and interruption criteria, or persistent grade 2 or higher toxicity for more than 2 weeks. Following successful completion of the first dose level, patients will increase abemaciclib to the -1 dose level (100 mg BID) for 2 weeks. Patients without toxicity leading to drug hold or reduction will then increase to the final dose level (full dose, as per FDA approved schedule), to be used for the remainder of the planned 2 years of abemaciclib therapy. The primary endpoint will be assessed after 12 weeks of adjuvant abemaciclib therapy. Patients will be followed for their entire course of abemaciclib to determine overall tolerability, completion rates, and compliance.

Blood, tissue, and stool will be collected from all participants for correlative studies. Participants will also participate in quality of life (QOL) surveys and symptom questionnaires at various time points through the course of study treatment.

1.2 Primary Objective

To evaluate a composite endpoint consisting of the 12-week rate of abemaciclib discontinuation for any reason and/or dose reduction and/or inability to reach or maintain the target standard dose for patients treated with a dose escalation strategy of abemaciclib in combination with adjuvant endocrine therapy for early-stage, high-risk HR+/HER2- breast cancer.

1.3 Secondary Objectives

- To assess the incidence of clinically meaningful diarrhea (G2-4 per NCI CTCAE)
- To assess the rate of abemaciclib discontinuation, dose reductions, and inability to reach or maintain the target dose at 6 months and 24 months of abemaciclib therapy
- To report treatment-related adverse events

1.4 Correlative Objectives

- To evaluate translational outcomes with genomic profiling of tissue samples from the primary tumor, ctDNA, and stool samples.
- To evaluate patient self-reported and general oncology health-related quality of life and to evaluate patient-reported outcomes
- To evaluate adherence to oral adjuvant therapy
- To evaluate abemaciclib dose intensity
- To evaluate features predictive of discontinuation, dose-reduction and inability to reach or maintain the target dose

2. BACKGROUND

2.1 Study Disease and Populations Affected by the Disease

Breast cancer is a leading cause of morbidity, mortality, and disability worldwide. Since 2020, breast cancer has been the most diagnosed malignancy worldwide, with a higher annual incidence rate than any other invasive tumor. Globally in 2020, 2.3 million new cases and more than 600,000 breast cancer-related deaths were reported.[1] In the United States, 290,560 new cases and 43,780 deaths from breast cancer were estimated for the year 2022, corresponding to an average lifetime risk of diagnosis of 13% for a woman.[2]

Breast cancer encompasses a heterogeneous group of biological entities, operationally recapitulated by clinical subtypes based on the expression of key prognostic-predictive histology markers.[3-5] Relevant prognostic markers that guide clinical decision-making include the hormone receptors (HR), including the estrogen receptor (ER) and progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Approximately 70% of invasive breast tumors exhibit ER and/or PR expression.[4,5]

Stage at presentation reflects public health cancer interventions, primarily the presence and population coverage of early detection programs, including mammographic screening.[1,6] In North American and Western European countries with established, population-based screening programs, more than 90% of people diagnosed with breast cancer are found to have early-stage disease. Accordingly, locally advanced tumors at presentation or *de novo* metastatic disease represent less than 10% of cases.[1,2,6]

Patients with HR-positive/HER2-negative early breast cancer can be effectively treated with multimodality therapeutic approaches, which may include surgery, radiation therapy, endocrine treatment, and in selected cases, chemotherapy and/or CDK4/6 inhibitors.[7,8] Selection of adjuvant treatment is informed by the individual risk of disease recurrence and death from breast cancer as well as patient preferences. Presently, the decision-making for patients with early breast cancer is based on the anatomical stage at presentation (i.e., TNM) and receptor status (ER, PR, HER2), along with key histologic features (e.g., tumor grade). For some patients, clinical decision-making is refined by genomic tools capable of better stratifying risk in the

setting for stage I and II breast cancers. For patients with lymph node-negative disease or with 1-3 pathologically involved axillary lymph nodes, the indication for chemotherapy is guided by genomic signatures. Adjuvant chemotherapy is indicated for patients with a "high" genomic score of disease recurrence, based on approved genomic tools. All patients with HR-positive disease have an indication for adjuvant endocrine treatment.[8,9]

In patients with higher risk of recurrence, the incorporation of the CDK4/6 inhibitor abemaciclib has demonstrated improvements in survival outcomes.[10] Patients with HR-positive/HER2-negative breast cancer and ≥4 pathologically involved axillary lymph nodes or with 1-3 positive nodes and other high-risk features (tumors larger than 5cm, poor differentiation/high grade [G3], high proliferation index [ki-67>20%]) derive meaningful clinical benefit with the addition of 2 years of adjuvant abemaciclib. The pivotal clinical trial monarchE showed that in this higher risk node-positive population, the addition of a planned 2 years of abemaciclib to ongoing adjuvant endocrine therapy leads to absolute improvement in invasive-disease free survival (iDFS) of 6.4% at 4-years, corresponding to a relative improvement of more than 33%.[11] Based on these data, abemaciclib was approved by the FDA in October 2021.

The use of abemaciclib has been associated with an increase in specific adverse events (AEs). The most common AEs observed in monarchE included (all grade): diarrhea (86.2%), neutropenia (45.2%) and fatigue (39.2%). In addition, treatment holds were experienced by 61.7% of patients, dose reductions in 43.3%, and discontinuations related to AEs were reported. At a follow-up of 27 months, 30.6% of patients discontinued abemaciclib for any reason, and 25.8% for reasons other than disease recurrence; discontinuations related to AEs were reported in 18.5% of cases.[12] The rates of discontinuation were highest in the first month (n=77, 2.8%). In the first 12 weeks of treatments, 10% of the patients taking abemaciclib had discontinued the drug (for any reason), and 35% experienced dose reductions. Two-third of the patients (n=324) discontinuing abemaciclib had experienced low-grade AEs (grade 1 and 2). In addition, 26% and 13.9% of patients experienced treatment-related holds or dose reductions within 1 and 2 months, respectively. At 6 months, approximately 15% of patients had discontinued abemaciclib for AEs, patient decision, or investigator decision (i.e., any reason).

Event	3-month	6-month	Overall
Dose modifications/ holds	In the 1st month: 25% In the 3rd month: 10%	In the 6th month: 5%	61.7%
Discontinuation (any reason)	10%	-	30.6%
Discontinuation (any reason other than breast cancer recurrence)	5% **	15%	25.8%
Discontinuation (due to AEs)	3% **	10%	18.5%
Dose reductions	35%	-	43.4%

***curve extrapolation. AE, adverse events. Data based on from Rugo H, Ann Onc 2022.[12]*

The main AEs leading to discontinuation were diarrhea (5.3%), fatigue (2%), and neutropenia (0.9%). These key AEs presented as moderate or severe in 7.7%, 19.1%, and 2.8% of patients, respectively.

Rates of diarrhea seen with abemaciclib in any setting are higher than those seen with the other available CDK4/6 inhibitors palbociclib and ribociclib. In monarchE, diarrhea was an early AE, with a median time to event of 8 days. Diarrhea led to dose reductions in 20.5% of patients (n=472) and holds in 22.9% (n=528). Anti-diarrheal medications were provided as needed to 78% of patients experiencing diarrhea, both for G1 (85.5%) and G3 (87.9%) severity. Dose discontinuation due to diarrhea was reported in 5% of the cases, mostly due to low-grade events, and was more frequent in the first 2 months of treatment. G1 diarrhea persisted at 1 year of treatment in patients still taking abemaciclib.[12]

The risk of disease recurrence in patients eligible for adjuvant abemaciclib (as per monarchE trial) is elevated, with recurrence rates of nearly 20% at 3 years and 30% at 5 years. Premature discontinuation of active adjuvant treatments may result in a detrimental effect on patient outcomes. Therefore, optimization of treatment tolerability and safety profile is a priority in the setting of higher risk disease. Optimized tolerance of therapy can result in better therapeutic adherence, ultimately allowing more patients to complete the planned adjuvant treatment plan and remain disease-free.

2.2 Abemaciclib

Abemaciclib is an inhibitor of CDK4 and CDK6. These kinases are activated upon binding to D-type cyclins. In ER+ breast cancer cell lines, cyclin D1 and CDK4/6 promote phosphorylation of the retinoblastoma (Rb) protein, cell cycle progression, and cell proliferation. *In vitro*, continuous exposure to abemaciclib inhibited Rb phosphorylation and blocked progression from G1 into S phase of the cell cycle, resulting in senescence and apoptosis. In breast cancer xenograft models, abemaciclib dosed daily without interruption as a single agent or in combination with antiestrogens resulted in reduction of tumor size.[13]

Pharmacokinetics

The pharmacokinetics of abemaciclib were characterized in patients with solid tumors, including metastatic breast cancer, and in healthy subjects. Following single and repeated twice daily dosing of 50 mg (0.3 times the approved recommended 150 mg dosage) to 200 mg of abemaciclib, the increase in plasma exposure (AUC) and Cmax was approximately dose proportional. Steady state was achieved within 5 days following repeated twice daily dosing, and the estimated geometric mean accumulation ratio was 2.3 (50% CV) and 3.2 (59% CV) based on Cmax and AUC, respectively.

Metabolism and Excretion

The absolute bioavailability of abemaciclib after a single oral dose of 200 mg is 45% (19% CV). The median Tmax of abemaciclib is 8.0 hours (range: 4.1-24.0 hours). A high-fat, high-calorie meal (approximately 800 to 1000 calories with 150 calories from protein, 250 calories from carbohydrate, and 500 to 600 calories from fat) administered to healthy subjects increased the AUC of abemaciclib plus its active metabolites by 9% and increased Cmax by 26%.

In vitro, abemaciclib was bound to human plasma proteins, serum albumin, and alpha-1-acid

glycoprotein in a concentration independent manner from 152 ng/mL to 5066 ng/mL. In a clinical study, the mean (standard deviation, SD) bound fraction was 96.3% (1.1) for abemaciclib, 93.4% (1.3) for M2, 96.8% (0.8) for M18, and 97.8% (0.6) for M20. The geometric mean systemic volume of distribution is approximately 690.3 L (49% CV). In patients with advanced cancer, including breast cancer, concentrations of abemaciclib and its active metabolites M2 and M20 in cerebrospinal fluid are comparable to unbound plasma concentrations. The geometric mean hepatic clearance (CL) of abemaciclib in patients was 26.0 L/h (51% CV), and the mean plasma elimination half-life for abemaciclib in patients was 18.3 hours (72% CV).

Hepatic metabolism is the main route of clearance for abemaciclib. Abemaciclib is metabolized to several metabolites primarily by cytochrome P450 (CYP) 3A4, with formation of N-desethylabemaciclib (M2) representing the major metabolism pathway. Additional metabolites include hydroxyabemaciclib (M20), hydroxy-N-desethylabemaciclib (M18), and an oxidative metabolite (M1). M2, M18, and M20 are equipotent to abemaciclib and their AUCs accounted for 25%, 13%, and 26% of the total circulating analytes in plasma, respectively.

After a single 150 mg oral dose of radiolabeled abemaciclib, approximately 81% of the dose was recovered in feces and approximately 3% recovered in urine. The majority of the dose eliminated in feces was metabolites.

<u>Specific Populations</u>

Age, Gender, and Body Weight
Based on a population pharmacokinetic analysis in patients with cancer, age (range 24-91 years), gender (134 males and 856 females), and body weight (range 36-175 kg) had no effect on the exposure of abemaciclib.

Patients with Renal Impairment
In a population pharmacokinetic analysis of 990 individuals, in which 381 individuals had mild renal impairment (60 mL/min ≤ CLcr <90 mL/min) and 126 individuals had moderate renal impairment (30 mL/min ≤ CLcr <60 mL/min), mild and moderate renal impairment had no effect on the exposure of abemaciclib [see Use in Specific Populations (8.6)]. The effect of severe renal impairment (CLcr <30 mL/min) on pharmacokinetics of abemaciclib is unknown.

Patients with Hepatic Impairment
Following a single 200 mg oral dose of abemaciclib, the relative potency adjusted unbound AUC0-INF of abemaciclib plus its active metabolites (M2, M18, M20) in plasma increased 1.2-fold in subjects with mild hepatic impairment (Child-Pugh A, n=9), 1.1-fold in subjects with moderate hepatic impairment (Child-Pugh B, n=10), and 2.4-fold in subjects with severe hepatic impairment (Child-Pugh C, n=6) relative to subjects with normal hepatic function (n=10) [see Use in Specific Populations (8.7)]. In subjects with severe hepatic impairment, the mean plasma elimination half-life of abemaciclib increased to 55 hours compared to 24 hours in subjects with normal hepatic function.

<u>*In Vitro* Studies</u>

Transporter Systems
Abemaciclib and its major active metabolites inhibit the renal transporters OCT2, MATE1, and MATE2-K at concentrations achievable at the approved recommended dosage. The observed serum creatinine increase in clinical studies with abemaciclib is likely due to inhibition of tubular secretion of creatinine via OCT2, MATE1, and MATE2-K. Abemaciclib and its major metabolites at clinically relevant concentrations do not inhibit the hepatic uptake transporters OCT1, OATP1B1, and OATP1B3 or the renal uptake transporters OAT1 and OAT3.

Abemaciclib is a substrate of P-gp and BCRP. Abemaciclib and its major active metabolites, M2 and M20, are not substrates of hepatic uptake transporters OCT1, organic anion transporting polypeptide 1B1 (OATP1B1), or OATP1B3. Abemaciclib inhibits P-gp and BCRP. The clinical consequences of this finding on sensitive P-gp and BCRP substrates are unknown.

CYP Metabolic Pathways
Abemaciclib and its major active metabolites, M2 and M20, do not induce CYP1A2, CYP2B6, or CYP3A at clinically relevant concentrations. Abemaciclib and its major active metabolites, M2 and M20, down regulate mRNA of CYPs, including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP3A4. The mechanism of this down regulation and its clinical relevance are not understood. However, abemaciclib is a substrate of CYP3A4, and time-dependent changes in pharmacokinetics of abemaciclib as a result of autoinhibition of its metabolism was not observed.

P-gp and BCRP Inhibitors
In vitro, abemaciclib is a substrate of P-gp and BCRP. The effect of P-gp or BCRP inhibitors on the pharmacokinetics of abemaciclib has not been studied.

Clinical Studies

MONARCH1

MONARCH 1 (NCT02102490) was a single-arm, open-label, multicenter study in women with measurable HR-positive/HER2-negative metastatic breast cancer whose disease progressed during or after endocrine therapy, had received a taxane in any setting, and who received 1 or 2 prior chemotherapy regimens in the metastatic setting. A total of
132 patients received 200 mg abemaciclib orally twice daily on a continuous schedule until development of progressive disease or unmanageable toxicity. Patient median age was 58 years (range, 36-89 years), and the majority of patients were White (85%). Patients had an Eastern Cooperative Oncology Group performance status of 0 (55% of patients) or 1 (45%). The median duration of metastatic disease was 27.6 months. Ninety percent (90%) of patients had visceral metastases, and 51% of patients had 3 or more sites of metastatic disease. Fifty-one percent (51%) of patients had had one line of chemotherapy in the metastatic setting. Sixty-nine percent (69%) of patients had received a taxane-based regimen in the metastatic setting and 55% had received capecitabine in the metastatic setting. At the 12-month final analysis, the ORR was 19.7%, clinical benefit rate was 42.4%, median progression-free survival (PFS) was 6.0 months, and median overall survival (OS) was 17.7 months. The most common treatment-related adverse

events of any grade were diarrhea (90.2%), increased creatinine (98.5%), leukopenia (90.8%), neutropenia (87.7%), anemia (68.5%), fatigue (65.2%), and nausea (64.4%). Discontinuations due to adverse events were infrequent (7.6%).[14]

MONARCH2

MONARCH 2 (NCT02107703) was a randomized, placebo-controlled, multicenter study in women with HR-positive/HER2-negative metastatic breast cancer in combination with fulvestrant in patients with disease progression following endocrine therapy who had not received chemotherapy in the metastatic setting. Randomization was stratified by disease site (visceral, bone only, or other) and by sensitivity to prior endocrine therapy (primary or secondary resistance). Primary endocrine therapy resistance was defined as relapse while on the first 2 years of adjuvant endocrine therapy or progressive disease within the first 6 months of first line endocrine therapy for metastatic breast cancer.

A total of 669 patients were randomized to receive abemaciclib or placebo orally twice daily plus intramuscular injection of 500 mg fulvestrant on days 1 and 15 of cycle 1 and then on day 1 of cycle 2 and beyond (28-day cycles). Pre/perimenopausal women were enrolled in the study and received the gonadotropin-releasing hormone agonist goserelin for at least 4 weeks prior to and for the duration of the study. Patients remained on continuous treatment until development of progressive disease or unmanageable toxicity. Patient median age was 60 years (range, 32-91 years), and 37% of patients were older than 65. The majority were White (56%), and 99% of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Twenty percent (20%) of patients had de novo metastatic disease, 27% had bone only disease, and 56% had visceral disease. Twenty-five percent (25%) of patients had primary endocrine therapy resistance. Seventeen percent (17%) of patients were pre- or perimenopausal.

Abemaciclib plus fulvestrant significantly extended PFS versus fulvestrant alone (median PFS 16.4 months vs. 9.3 months; $p < 0.001$). In patients with measurable disease, abemaciclib plus fulvestrant achieved an ORR of 48.1% compared to 21.3% with fulvestrant alone. The most common adverse events in the abemaciclib plus fulvestrant arm were diarrhea (86.4%), neutropenia (46.0%), nausea (45.1%), and fatigue (39.9%).[15] Abemaciclib plus fulvestrant produced a median OS of 46.7 months, versus 37.3 months for fulvestrant alone ($p = 0.01$). Improvement in OS was consistent across all stratification factors. Median time to second disease progression (23.1 months vs. 20.6 months), median time to chemotherapy (50.2 months vs. 22.1 months), and median chemotherapy-free survival (25.5 months vs. 18.2 months) were significantly improved in the abemaciclib plus fulvestrant versus fulvestrant monotherapy arm.[16]

MONARCH3

MONARCH 3 was a double-blind, randomized phase III study of abemaciclib plus a non-steroidal aromatase inhibitor (NSAI) versus NSAI alone in 493 postmenopausal women with HR+/HER2- advanced breast cancer who had not received any prior systemic therapy in the advanced setting. Patients received abemaciclib (150 mg twice daily continuous schedule) or placebo plus either 1 mg anastrozole or 2.5 mg letrozole daily. The primary endpoint was

investigator-assessed PFS; secondary objectives included response evaluation and safety. A planned interim analysis occurred after 189 events.

The interim analysis occurred after 194 PFS events (108 [32.9%] in the abemaciclib arm and 86 [52.1%] in the placebo arm). The median follow-up was 17.8 months. The study met its primary endpoint with an observed investigator-assessed PFS hazard ratio of 0.54 (p = 0.000021). The median was not reached in the abemaciclib arm and was 14.7 months in the placebo arm. In the ITT population, the ORR was 48.2% in the abemaciclib arm and 34.5% in the placebo arm (p = 0.002). In patients with measurable disease, the ORR was 59% in the abemaciclib arm and 44% in the placebo arm (p = 0.004). In the abemaciclib arm, diarrhea was the most frequent adverse event (81.3%), but was mostly grade 1 (44.6%). Comparing abemaciclib vs. placebo, the most frequent grade 3 or 4 adverse events were neutropenia (21.1% vs. 1.2%), diarrhea (9.5% vs. 1.2%), and leukopenia (7.6% vs. 0.6%).[17]

At the final PFS cutoff, 138 events had occurred in the abemaciclib arm (42.1%) and 108 had occurred in the placebo arm (65.5%). The median follow-up was 26.73 months. The median investigator-assessed PFS was 28.18 months in the abemaciclib arm vs. 14.76 months in the placebo arm (p = 0.000002). Analysis of PFS across patient subgroups revealed that all patient subgroups benefitted from the addition of abemaciclib to NSAI.[18]

monarchE

monarchE (NCT03155997) was a randomized, open-label, phase III trial of adjuvant abemaciclib plus ET or ET alone in patients with stage II-III HR+/HER2- breast cancer with high risk of recurrence. High risk was defined as four or more pathologically involved axillary lymph nodes or 1-3 pathologically involved axillary lymph nodes with at least one of the following features: tumor size ≥ 5 cm, histologic grade 3, or centrally assessed Ki-67 ≥ 20%. A total of 5,637 patients were randomized 1:1 to receive two years of abemaciclib 150 mg twice per day in combination with standard adjuvant ET or ET alone. At a pre-planned interim efficacy analysis, there were 323 iDFS events observed, with 136 (4.8%) in the abemaciclib arm and 187 (6.6%) in the control arm. Abemaciclib plus ET demonstrated a significant improvement in iDFS versus ET alone (p = 0.01; HR: 0.75; 95% CI: 0.60 – 0.93). The 2-year iDFS was 92.2% in the abemaciclib arm and 88.7% in the ET alone arm. The addition of abemaciclib to ET also resulted in a significant improvement in distant relapse-free survival (DRFS; nominal p = 0.01; HR: 0.72; 95% CI: 0.56 – 0.92), with 2-year DRFS rates of 93.6% in the abemaciclib arm versus 90.3% in the ET alone arm.[19]

At the primary outcome analysis of monarchE, after a median follow-up of approximately 19 months in both arms, 395 iDFS events had been observed in the ITT population, with 163 (5.8%) observed in the abemaciclib arm and 232 (8.2%) in the control arm (log-rank P = 0.0009; HR: 0.713; 95% CI: 0.583 – 0.871). The 2-year iDFS was 92.3% in the abemaciclib arm and 89.3% in the control arm. The 2-year DRFS was 93.8% in the abemaciclib arm and 90.8% in the control arm (log-rank P = 0.0009; HR: 0.687; 95% CI: 0.551 – 0.858).[20]

After a median follow-up of 27 months on the monarchE trial, the combination of abemaciclib plus ET continued to result in significantly improved iDFS compared to ET alone (HR: 0.70;

95% CI: 0.59 – 0.82; nominal p < 0.0001). The 3-year iDFS was 88.8% on the abemaciclib arm and 83.4% on the control arm. In addition, the DRFS was significantly improved on the abemaciclib arm compared to the control arm (HR: 0.69; 95% CI: 0.57 – 0.83; nominal p < 0.0001). The 3-year DRFS was 90.3% on the abemaciclib arm and 86.1% on the control arm.[10]

After a median follow-up of 42 months on the monarchE trial, 835 iDFS events were observed in the ITT population, including 336 (12.0%) in the abemaciclib plus ET group and 499 (17.6%) in the ET group. The addition of abemaciclib to adjuvant ET continued to result in significantly improved iDFS compared to ET alone (HR: 0.664; 95% CI: 0.578 – 0.762; nominal p < 0.0001). The 4-year iDFS was 85.8% in the abemaciclib plus ET group and 79.4% in the ET group. The addition of abemaciclib to ET also continued to result in a significant improvement in DRFS compared to ET alone (HR: 0.659; 95% CI: 0.567 – 0.767; nominal p < 0.0001). The estimated 4-year DRFS was 88.4% in the abemaciclib plus ET arm and 82.5% in the ET group.[11]

Patients will receive a dose-escalation schedule of abemaciclib, starting at 50mg PO BID and escalating to 150mg BID, the current approved full dose for this treatment, as described in section 5. For further information on the clinical safety of abemaciclib, please see the current Investigator's Brochure.

2.3 Hormonal Therapy

Standard anti-estrogen hormonal therapy agents will be used on trial, which are: tamoxifen (selective estrogen receptor modulator); anastrozole and letrozole (non- steroidal aromatase inhibitors); exemestane (steroidal aromatase inhibitor); and LHRH agonists.

2.4 Rationale

Treatment schedules of initial dose-escalation have been implemented in the past to optimize the tolerability of cancer treatments and enhance the therapeutic adherence by improving the safety profiles of anticancer drugs. Supportive care or dose-escalation strategies have been explored for other targeted breast cancer agents that cause potentially exposure-limiting toxicity.[21,22] In breast cancer treatment, a dose-escalation strategy has proved effective in reducing clinically meaningful AEs in the adjuvant setting for patients with HER2-positive breast cancer taking neratinib. Patients enrolled in the pivotal trial ExteNET trial experienced a rate of grade 3 diarrhea of 40%, with 17% reported related discontinuation.[23] The CONTROL study evaluated methods to reduce toxicity and optimize therapeutic adherence. In this trial, use of a dose-escalation schedule reduced the rate of observed grade 3 diarrhea to 15%, with only 3% of patients discontinuing therapy for diarrhea.[22] Presently, a strategy of dose-escalation for neratinib is included among the clinical guideline recommendations and reported on the neratinib prescribing information as a method to improve therapeutic adherence and ultimately result in better outcomes.[8]

A dose escalation approach has also been applied for everolimus, in a study presented by the German West Group. The DESIREE trial was a phase 2 trial evaluating the tolerability of an

induction dose-escalation of everolimus in patients with metastatic breast cancer.[24] Patients were randomized to the standard schedule of 10mg everolimus daily versus a modified, dose-escalation schedule, with an initial dose of 2.5mg and a progressive weekly escalation up to the full dose by the fourth week, to assess the impact on the occurrence of mucositis, a chief and clinically meaningful AEs with this treatment. The trial showed that use of the modified schedule significantly reduced the occurrence of grade 2 or higher mucositis (28.8 vs 46.1%, p=0.039), with no reduction of the overall relative total dose intensity, and fewer observed treatment discontinuations in the first 12 weeks (6.3 vs 15.8%, p=0.073).

Modified treatment schedules with initial dose-escalation have also been implemented in other disease settings. In patients with advanced hepatocellular carcinoma, an AE dose-adapted schedule of sorafenib resulted in prolonged drug exposure, ultimately resulting in better survival outcomes.[25] Building on this experience, a trial in the second line setting with regorafenib dose escalation is currently ongoing for patients with liver cancer (NCT04476329).

Some abemaciclib-related AEs may reflect off-target activities of the drug. The three most common AEs leading to treatment discontinuations were diarrhea, neutropenia, and fatigue.[10] The exact mechanism of abemaciclib-induced diarrhea has been explored in the preclinical setting. A murine model assessing the gastrointestinal safety of abemaciclib showed unique histopathologic findings, with distinctive changes characterized by proliferation of crypt cells, loss of goblet cells, poorly differentiated and degenerating enterocytes with loss of microvilli, and mucosal inflammation.[26] Abemaciclib-induced changes seemed to be mediated by secondary targets other than CDK4/6, such as the inhibition of GSK3β and consequential activation of the Wnt/β-catenin pathway, which have been associated with rare genetic syndromes with chronic diarrhea.[27] The exact mechanism of abemaciclib-induced neutropenia has also been the object of investigation as a class effect of CDK4/6 inhibitors. Experimental models show that the inhibition of CDK6 can impact CDK6-mediated erythroid development, myeloid differentiation, and hematopoietic stem cell activation, leading to reversable hematological AEs, including neutropenia.[28] Therefore, a dose-dependent effect is suggested, as these AE result from an on- or off-target pharmacological effect of abemaciclib. Tissue-level adaptations may occur as a result of progressive exposure to the drugs in non-cancer cells, ultimately improving the patient tolerability and reducing the burden of AEs.

Of note, a brief initial dose-escalation is not anticipated to expose a patient to inferior outcomes. The initial dose in this study is 50mg BID of abemaciclib, a dose used in clinical practice after 2 levels of dose reduction for patients not able to tolerate the full dose, based on the present FDA label and the clinical trials, therefore expected to exert therapeutic effects. Previous experience with the use of CDK4/6 inhibitors including with abemaciclib in the metastatic setting showed no detriment on PFs and OS for patients who had to dose-reduce.[12,29-35] Also, the pivotal monarchE clinical trial included patients who had received their definitive breast cancer surgery up to 16 months before enrollment, and up to 12 weeks of previous adjuvant endocrine therapy. Accordingly, a 4-week dose-escalation regimen concurrently to hormone therapy is not anticipated to affect the overall treatment effect.

The aim of the TRADE study is to test a strategy of dose-escalation of adjuvant abemaciclib to reduce treatment discontinuations and/or dose reductions through improvement of drug

tolerability and safety profile.

2.5 Correlative Studies Background

2.5.1 Circulating tumor DNA (ctDNA)

Correlative samples will be collected to identify potential ctDNA-related biomarkers of prognosis, including the presence of ctDNA at the time of abemaciclib initiation, the ctDNA dynamic changes during the early phase of treatment, and the ctDNA clearance. In addition, for patients with detectable ctDNA, genomic sequencing will be performed. ctDNA analyses will be relevant to identify predictors of benefit and resistance to adjuvant abemaciclib that can be identified in the earliest phase of the treatment, potentially identifying additional treatment strategies to improve outcomes for patients at higher risk of recurrence. The analysis will focus on potential variables of early benefit and resistance to adjuvant abemaciclib, including genes related to CDK4/6 inhibitor mechanisms of action, immune function, inflammatory responses, microenvironment, tumor metabolism, stress response, and encompassing the spectrum of alterations associated or potentially associated with endocrine resistance, such as ESR1, ARID1A, or mediated by IGFR, HER2, FGFR 1-3, c-Myc, or TP53. Optionally, archival FFPE specimens from breast biopsy or surgery will be collected and analysed by genomic profiling as well.

2.5.2 Stool Analyses

Participants will be asked to provide stool samples. Stool-based analyses will enhance the current knowledge of the biochemical alterations that abemaciclib can induce on the feces, which to date has been only reported in animal models. Better identification of physic-chemical characteristics of the stool and the assessment of key markers of gut inflammation, for example calprotectin, lactoferrin and the presence and type of immune cells, will increase our knowledge of potential mechanisms of pathogenesis of key side effects of abemaciclib, including the classification of the diarrhea types, therefore helping to tailor ad hoc supportive treatments as opposed to non-specific anti-diarrheal approaches. For instance, if the GSK-3β/Wnt-βcatenin off-target effect is key to the etiology of abemaciclib-related diarrhea, by facilitation of proliferation and virulence of selected pathogens or exerting secretory stimulation on the gut epithelium, a tailored approach may be designed. In addition, evaluation of microorganisms to analyse the intestinal microbiota through metagenomics will be performed. *In vitro* evidence suggests that abemaciclib induces a T-cell inflammatory response that has been poorly characterised in humans.[36] CDK4/6 inhibitors have been shown to enhance expression of MHC-I in preclinical models of breast cancer, leading to the activation of interferon responses.[37] In addition, CDK4/6 inhibitors can increase HLA ligands with a higher chance to be recognized by T cells, namely antigen presentation, that may render breast cancer more immunogenic.[38] Immune modulation seems to be exerted through T-cell specific properties of CDK4/6 inhibitors, yielding increased T-cell persistence and immunologic memory.[39] The immune-modulatory properties of abemaciclib may ultimately be affected by characteristics and treatment-induced changes of the gut microbiota. It has been reported, for example, that benefit from neoadjuvant treatment for breast cancer can be informed by metagenomics: low diversity/low abundances, a specific metagenomic composition with

decreased butyrate-producing and indole-propionic acid-producing bacteria and increased potential pathobionts are key features associated with response to neoadjuvant chemotherapy.[40] Overall, metagenomics can integrate knowledge of the activities of abemaciclib in the early setting, including as it pertains immunomodulatory properties, and how it can be predicted through microbiota-based biomarkers.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Stage II or III node-positive HR+/HER2- breast cancer per local laboratory and/or provider assessment.

3.1.2 Eligible participants must be appropriate candidates for adjuvant abemaciclib, per assessment of their treating physician.

3.1.3 Participants must be candidates for adjuvant endocrine therapy, which may have started before or at time of entry onto the trial. Patient may be receiving adjuvant aromatase inhibitor or tamoxifen, +/- ovarian suppression.

3.1.4 Participants must have undergone definitive surgery of the primary breast tumor(s) within 16 months of study entry.

3.1.5 At least 21 days must have elapsed between last dose of chemotherapy and registration. Participants who previously received chemotherapy must have recovered (Common Terminology Criteria for Adverse Events [CTCAE] Grade ≤1) from the acute effects of chemotherapy except for residual alopecia or Grade 2 peripheral neuropathy prior to randomization.

3.1.6 At least 14 days must have elapsed between end of radiotherapy and day 1 of treatment with abemaciclib. Participants who received prior radiotherapy must have completed and fully recovered from the acute effects of radiotherapy. No radiotherapy should be planned to occur during study therapy.

3.1.7 At least 14 days must have elapsed since most recent breast surgery prior to registration and patient has recovered from side effects of prior surgery.

3.1.8 Bilateral or multifocal/multicentric breast cancers that meet eligibility criteria are allowed.

3.1.9 ECOG performance status 0-1

3.1.10 Men and women with any menopausal status ≥18 years of age

3.1.11 Adequate organ function as defined below:

- Absolute neutrophil count (ANC) ≥ 1500 x 10^9/L

- Platelets ≥ 100 x 10^9/L
- Hemoglobin ≥ 8g/dL; 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 after the erythrocyte transfusion.
- Bilirubin ≤ 1.5 x ULN. For patients with Gilbert syndrome, the limit is ≤ 2 x institutional ULN AND direct bilirubin within the normal range of normality.
- AST/ALT ≤ 3 x institutional ULN

3.1.12 Premenopausal women must have a negative serum or urine pregnancy test. Pregnancy testing does not need to be pursued in female patients who are:
- Age > 60 years; or
- Age < 60 with intact uterus and amenorrhea for 12 consecutive months or more AND FSH/estradiol levels within postmenopausal range; or
- Status-post bilateral oophorectomy, total hysterectomy, or bilateral tubal ligation.

3.1.13 Women of child-bearing potential and men with partners of childbearing potential must be willing to employ one highly effective form of nonhormonal contraception (with the exception of hormonal IUDs) or two effective forms of nonhormonal contraception by the patient and/or partner and continue its use for the duration of the study treatment and for 3 months after the last dose of abemaciclib.

3.1.14 Subject must be able to swallow and retain oral medication.

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

3.1.16 Non-English-speaking patients are eligible but will be exempt from patient-completed questionnaires.

3.2 Exclusion Criteria

3.2.1 Prior treatment with any CDK4/6 inhibitor.

3.2.2 Patients with node-negative breast cancer are not eligible for the trial.

3.2.3 Concurrent therapy with other investigational agents.

3.2.4 History of allergic reactions attributed to abemaciclib or similar chemical or biologic composition or excipients.

3.2.5 Participants with a history of malignancy are ineligible except in the following circumstances:
- Individuals with a history of invasive breast cancer are not eligible unless they have been disease-free for a minimum of five years.
- Individuals with a malignancy history other than invasive breast cancer are eligible if they have no active malignancy and are deemed by the investigator to be at low risk for

recurrence of that malignancy.

3.2.6 Individuals with the following cancer history are eligible: adequately treated non-melanoma skin cancers, curatively treated in situ cancer of the cervix, ductal carcinoma in situ (DCIS) of the breast, stage 1 grade 1 endometrial carcinoma. Other exceptions may exist following review with the sponsor-investigator

3.2.7 Serious and/or uncontrolled preexisting medical condition(s) that, in the judgment of the investigator, would preclude participation in this study (for example, interstitial lung disease, severe dyspnea at rest or requiring oxygen therapy, severe renal impairment [e.g. estimated creatinine clearance <30ml/min], history of major surgical resection involving the stomach or small bowel, or preexisting uncontrolled Crohn's disease or ulcerative colitis or a preexisting chronic condition resulting in baseline Grade 2 or higher diarrhea) or other conditions that in the opinion of the investigator limit compliance with study requirements.

3.2.8 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.

3.2.9 Any of the following due to teratogenic potential of the study drugs:

- Pregnant women
- Nursing women
- Women of childbearing potential who are unwilling to employ adequate contraception (condoms, diaphragms, IUDS, surgical sterilization, abstinence, etc). Hormonal birth control methods are not permitted.
- Men who are unwilling to employ adequate contraception (condoms, surgical sterilization, abstinence, etc).

3.2.10 Receipt of an experimental treatment in a clinical trial within the last 30 days or 5 half-lives, whichever is longer, prior to enrollment, or is currently enrolled in any other type of medical research (for example: medical device) judged by the sponsor-investigator not to be scientifically or medically compatible with this study.

3.2.11 Active systemic bacterial infection (requiring intravenous [IV] antibiotics at time of initiating study treatment) or invasive/ systemic fungal infection.

3.2.12 For patients with known HIV infection, CD4 baseline count should be evaluated: patients with a CDK count ≥ 350 cells/uL can be enrolled. Participants should be on established anti-retroviral therapy (ART) for at least four weeks and have an HIV viral load less than 400 copies/mL prior to enrollment. Potential pharmacological interactions of the ART with abemaciclib and endocrine therapy must be reviewed, particularly for the effects on CYP3A4.

3.2.13 Patients with active or chronic Hepatitis B or C are eligible provided they meet liver function laboratory criteria and cannot be on any medication with a known interaction

with the study agents.

3.2.14 Participants receiving any medications or substances that are strong inhibitors or inducers of CYP3A4, including selected herbals (e.g., hypericum) and food (e.g., grapefruit) known for pharmacological interactions, cannot be enrolled, due to interference with the dose-escalation, unless the food or supplement has been discontinued at least after an interval equivalent to 3-5 half-lives of the inhibitor.

3.2.15 Participants with planned activities that would result in a dose hold or modification for non-tolerability related reasons in the first 12 weeks of protocol therapy, including elective surgeries and travel.

3.3 Inclusion of Women and Minorities

Subjects of any gender, of all races and ethnic groups are eligible for this trial. In addition, participants with disabilities, those at the extremes of weight, and those whose preferred language is not English are also eligible for this trial.

Breast cancer is a common malignancy among women, most common in non-Hispanic White women, second most common among non-Hispanic Black women, and least common among Hispanic women (SEER Cancer Stat Facts: Female Breast Cancer). Breast cancer is rare in men of any race. The study team will prioritize ensuring that this trial is presented to all eligible patients, regardless of demographics. We will make every effort to provide a translated consent form in addition to publicizing the trial to all Dana-Farber/Harvard Cancer Center patients.

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 any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention 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-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Principal Investigator (PI) of the registering site. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute by the Project Manager. The required forms listed in Section 4.4 should be emailed to the Project Manager.

Following registration, participants should begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator. If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. The Project Manager should be notified of cancellations as soon as possible and the project manager will take the subject off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and emailed to the Project Manager at [redacted]:

- Completed DF/HCC Eligibility Checklist
- Signed participant consent form
- HIPAA authorization form (if separate from the main consent form)
- Clinic visit note documenting history and physical exam, ECOG PS, and vital signs
- Copy of required laboratory tests including: Hematology and Chemistry
- Local pathology report with documentation of ER, PR, and HER2 status
- Confirmation of request or availability of archival tissue

To complete the registration process, the Project Manager will

- Follow the DF/HCC Policy REGIST-101and register the participant on the protocol
- Email the study team at the participating site with the participant study number, and registration confirmation

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

5. TREATMENT PLAN

TRADE is a single-arm, prospective, phase 2 study of the tolerability of a dose-escalation strategy of adjuvant abemaciclib for high-risk early-stage HR+/HER2- breast cancer. The treatment cycle is defined as 28 consecutive days of treatment with abemaciclib and endocrine treatment, regardless of dose holds and reductions. Treatment will be administered on an outpatient basis.

5.1 Treatment Regimen

Participants enrolled in the TRADE study will receive a dose-escalation schedule of abemaciclib for the first cycle (28 days) on study. Participants will start treatment at the -2 dose level with 50-mg twice daily for 14 days. If this dose is tolerable, then treatment will escalate to the -1 dose level, 100-mg twice daily for 14 days. If this is tolerable, then the patient will escalate to the full dose as per standard of care, 150-mg twice daily. See section 5.3 for the criteria to dose-escalate. Participants who are unable to escalate within the first cycle of therapy may remain on study at a tolerable dose (as determined by treating investigator) for the remainder of 2 years of adjuvant therapy.

Abemaciclib will be administered with concurrent ongoing adjuvant endocrine therapy of provider choice.

The dose escalation schedule will be administered as described in the below table.

Days of treatment	**Dose of abemaciclib**	**Level of dose**	**Endocrine treatment**
Day 1-14	50mg PO, BID	-2	Standard continuous daily adjuvant ET of physician's choice may be initiated prior to registration.
Day 15-28	100 mg PO, BID	-1	
Day 29-84 (to complete 2 years)	150 mg PO, BID	Full dose	

D, days. PO, per os. BID, twice a day. ET, endocrine treatment.

Day 1 of the treatment is defined as the first day taking abemaciclib. Patients may have initiated provider choice endocrine treatment earlier. Patients will be monitored on study until the completion of the planned 2-year course of abemaciclib, or unless discontinuation criteria are fulfilled.

Endocrine therapy can consist of aromatase inhibitor (letrozole, anastrozole or exemestane) or tamoxifen administrated based on standard schedules, with or without ovarian function suppression (LHRH modulators). Selection of endocrine therapy is at the discretion of the treating provider and can be changed or modified during the course of the study, as appropriate based on tolerability and clinical practice.

Participants will be required to maintain a medication diary for abemaciclib and endocrine therapy dosing as well as return all bottles of abemaciclib at each study visit. Accountability of the number of pills of abemaciclib prescribed versus taken will be maintained by the study team, for the purpose of the therapeutic adherence and compliance analyses. Drug accountability is not required for endocrine therapy, but participants should be counseled by the study team on the importance of adherence in combination with review of drug diary at each study visit.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

If screening assessments were completed ≤ 7 days prior to cycle 1 day 1, laboratory tests do not need to be repeated on cycle 1 day 1 and the screening laboratory values can be used as the cycle 1 day 1 (C1D1) values.

If C1D1 laboratory testing is performed, the following laboratory criteria must be met:

- Absolute neutrophil count (ANC) ≥ 1500 /mm^3
- Platelets ≥ 100 x 10^9/L
- Hemoglobin ≥ 8g/dL
- Bilirubin ≤ 1.5 x institutional ULN. For patients with Gilbert syndrome, the limit is ≤ 2 x institutional ULN AND direct bilirubin within the normal range.
- AST/ALT ≤ 3 x institutional ULN

Dose Modifications and management of specific toxicities considered at least possibly related to the study regimen are outlined in **Section 6**.

5.3 Criteria to Dose Escalate

Patients will escalate to the next abemaciclib dose level if the current dose is tolerable.

Patients can dose escalate based on these criteria in the opinion of the treating investigator:

- No ongoing grade 3/4 hematologic toxicity.
- No ongoing grade 3/4 non-hematologic toxicity.
- No ongoing non-hematologic grade 2 toxicity related to abemaciclib that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1.
- No persistent-recurrent (lasting ≥ 14 days) grade 2 diarrhea that does not resolve with maximal supportive measures, or requires hospitalization, or grade 3/4.
- No interstitial lung disease/ pneumonitis grade 2-4.
- Criteria for dose modification and dose-interruption in Section 6 do not apply.

5.4 Agent Administration

5.4.1 Abemaciclib

Abemaciclib is provided as 50-mg film-coated tablets. Abemaciclib should be taken at the same time every 12 hours ±4 hours with a glass of water. Abemaciclib must be taken with at least 6 hours separating doses. Tablets must be swallowed intact, as provided, and they are not intended to be chewed or crushed. Abemaciclib can be taken with or without food. Doses that would occur outside of this timeframe should be considered missed and should not be administered; participants should be advised that they should never double dose. If a participant vomits following a dose, the dose should not be re-taken, instead the participant should continue with their next regularly scheduled dose as clinically appropriate.

On days when patient is scheduled for a clinic visit, the patient may take the oral medications at home or in the clinic.

At enrollment, patients should receive instructions on the prompt management of diarrhea and provided with antidiarrheal medications (e.g., loperamide). In the event of diarrhea, supportive care measures should be initiated as early as possible.

5.4.2 Endocrine Therapy

Administration of endocrine therapy is performed on an outpatient, self-administration basis according to local requirements and local standard practice. Endocrine therapy can be taken either before or after abemaciclib.

The following standard endocrine treatment agents are allowed:

- Tamoxifen
- Non-steroidal aromatase inhibitors (anastrozole, letrozole)
- Steroidal aromatase inhibitor (exemestane)
- LHRH agonists in combination with tamoxifen or AI

Rotation of AI therapy among agents is allowed, as is change to AI from tamoxifen or change to tamoxifen from AI.

Patients receiving LHRH agents can be checked for ovarian suppression, based on local practice and international guidelines, including laboratory evaluation of plasma estrogen and FSH/LH, as appropriate. If a premenopausal or male patient is receiving LHRH agonist and AI, it is recommended that she/he receives monthly injections rather than every-3-month depot injections.

Expected toxicities and potential risks can be obtained from respective local package inserts and/or local standard operating procedures and standard informational handouts.

Missed doses of endocrine therapy should not be made up.

5.5 General Concomitant Medication and Supportive Care Guidelines

CYP3A inhibitors. Avoid concomitant use of strong CYP3A inhibitors (for example, voriconazole) and use caution with co-administered moderate (for example, ciprofloxacin) or weak (for example, ranitidine) CYP3A inhibitors. If coadministration with a strong CYP3A inhibitor is unavoidable, patients cannot continue in the trial if they are in the dose-escalation phase. Avoid grapefruit or grapefruit juice. If a CYP3A inhibitor is discontinued, abemaciclib can be started after 3-5 half-lives of the inhibitor.

- If coadministration with a strong CYP3A inhibitor is unavoidable, patients cannot be

enrolled. This trial has dose-escalation steps aiming at testing increasing doses of abemaciclib that in case of concomitant drugs affecting the metabolism would be unreliable and/or unpredictable. Patients in the dose-escalation phase should avoid concomitant intake of CYP3A4 strong inhibitors or inducers, including selected herbals (e.g., hypericum) and food (e.g., grapefruit) known for pharmacological interactions. No restriction is reported for co-administration of other drugs that are substrates of CYP enzymes.

- Only for patients who have completed the dose escalation phase, 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.

Abemaciclib is predominantly cleared by oxidative metabolism via CYP3A4.
Clinical drug interaction studies with a CYP3A inhibitor and CYP3A inducer significantly altered the PK of abemaciclib and its circulating major metabolites.
-CYP3A inducers: avoid concomitant use of CYP3A inducers and consider alternative agents.
-CYP3A inhibitors: avoid concomitant use of strong CYP3A inhibitors (for example, voriconazole) and use caution with co-administered moderate (for example, ciprofloxacin) or weak (for example, ranitidine) CYP3A inhibitors.

Concomitant administration of adjuvant bisphosphonates or denosumab is permitted.

Concomitant use of granulocyte-stimulating factors is not permitted.

Concurrent radiotherapy is not allowed nor anticipated in this trial. Patients must have completed radiation therapy > 14 days as per eligibility criteria.
Elective surgery, travel, or any other activity that would require a dose hold or modification within the first 12 weeks of protocol therapy is prohibited. Participants with known planned surgery or travel that would require a dose hold/modification during the first 12 weeks of protocol therapy are excluded from enrollment.

Elective surgery after 12 weeks of protocol therapy is permitted. Abemaciclib should be held for 1 week prior to surgery and 1-2 weeks after surgery and/or when adequate healing has occurred in the opinion of the treating investigator.

Appendix C presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

5.6 Criteria for Taking a Participant Off Protocol Therapy

Participants will be removed from the protocol therapy when one of the following criteria applies:

- Completion of 2 years of planned protocol therapy

- 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). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the participant's status must be updated in OnCore in accordance with REGIST-OP-1.

5.7 Duration of Follow Up

All subjects will be asked to return to the site for a final End-of-Treatment visit after last dose of protocol therapy. The End-of-Treatment visit must be performed within 30 days of final administration of study treatment. End-of-treatment assessments will not have to be repeated if the same assessments were performed within 7 days of this visit. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.8 Criteria for Taking a Participant Off Study

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

- Completion of study procedures and completion of 30-day safety follow-up period
- Withdrawal of consent to participate
- Death
- Lost to follow-up

Subjects who withdraw after signing the informed consent form (ICF) but before receiving study treatment will be replaced.

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1.

6. DOSING DELAYS/DOSE MODIFICATIONS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A

copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Abemaciclib Dose Modifications

Dose reductions should be made in 50 mg increments according to the dose level table below. For example, if a patient is receiving abemaciclib at 150 mg BID, dose level -1 would be 100 mg BID, dose level -2 would be 50 mg BID.

Patients will initially receive a modified, dose-escalation schedule of abemaciclib. The starting dose is 50 mg PO BID, which is dose level -2. Dose levels are described as:

Dose adjustments (level)	Oral dose
0 (full dose/ target dose)	150 mg BID (3 x 50 mg tablets, BID)
-1	100 mg BID (2 x 50 mg tablets, BID)
-2	50 mg BID (1 x 50 mg tablet, BID)

Abemaciclib to be taken twice daily with at least 6 hours between doses.

Dose escalation must be provided sequentially by 1 dose level. For patients taking 50 mg (starting dose), abemaciclib dose cannot be further reduced. If dose reduction is required below 50 mg, abemaciclib must be discontinued.

Patients will proceed to the next dose level only if they do not experience adverse events qualifying for dose-reduction and interruption criteria, or persistent grade 2 or higher toxicity for more than 2 weeks. (Please refer to section 5.0)

6.2 General Guidance for Toxicity Management

Abemaciclib may be held up to 28 days (1 cycle) continuously to permit sufficient time for recovery from toxicity. For patients not recovering from toxicity within 28 days, a delay >28 days is acceptable after discussion with sponsor-investigator. Unless otherwise specified, dose modifications for unacceptable adverse events after 12 weeks of protocol therapy are at the discretion of the treating investigator.

<u>Hematologic toxicities</u>
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.

CTCAE grade	Abemaciclib dose modifications
Grade 1 or 2	No dose modification is required

Grade 3	Suspend dose until toxicity resolves to ≤Grade 2. Dose reduction not required.
Grade 3 recurrent or Grade 4	Suspend dose until toxicity resolves to ≤Grade 2. Resume at next lower dose.

Diarrhea management

At enrollment, patients should receive instructions on the prompt management of diarrhea and provided with antidiarrheal medications (e.g., loperamide). In the event of diarrhea, supportive care measures should be initiated as early as possible. These include the following:

- At the first sign of loose stools, the patient should initiate antidiarrheal therapy (e.g. loperamide) and notify the investigator for further instructions and appropriate follow up.
- Loperamide may be taken 4 mg initially (e.g., 2 capsules of 2mg each), then 2 mg (e.g., 1 capsule of 2 mg) after each loose stool; dose should not exceed 16 mg/day (i.e., 8 capsule of 2mg) in 24h.
- Patients should also be encouraged to drink fluids (e.g., 8 to 10 glasses of clear liquids per day).

Patients with persistent G2 diarrhea lasting >2 weeks despite medical management will be unable to dose escalate, based on the **"Criteria to dose-escalate".**

At the first sign of loose stools, start treatment with antidiarrheal agents, such as loperamide.	
CTCAE Grade	**Abemaciclib dose modifications**
Grade 1	No dose modification
Grade 2	If toxicity does not resolve within 24h to ≤Grade 1, suspend dose until resolution. Dose reduction is not required.
Grade 2 that persists or recurs after resuming the same dose despite maximal supportive measures	Suspend until toxicity resolves ≤Grade 1. Resume at next lower dose.
Grade 3 or 4 or requires hospitalization.	Suspend until toxicity resolves ≤Grade 1. Resume at next lower dose.

Hepatic monitoring.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) elevation are considered as adverse drug reactions (ADR) with the use of abemaciclib.

CTCAE Grade	**Abemaciclib dose modifications**
Grade 1 (>ULN-3xULN if baseline was normal; 1.5-3 x baseline if baseline was abnormal)	No dose modification
Grade 2 (>3-5 x ULN if baseline was normal; >3-5 x baseline if baseline was abnormal)	No dose modification
Persistent or recurrent Grade 2 or Grade 3 (>5-	Suspend dose until toxicity resolved to

20 x ULN if baseline was normal; >5-20 x baseline if baseline was abnormal) without increase in total bilirubin above 2 x ULN	baseline or Grade 1. Resume at next lower dose.
Elevation in AST and/or ALT >3 x ULN, with total bilirubin above 2 x ULN.	Discontinue abemaciclib.
Grade 4 (> 20 x ULN if baseline was normal, >20 x ULN if baseline was abnormal)	Discontinue abemaciclib.

Hepatic testing including ALT, AST, alkaline phosphatase (ALP), and total bilirubin (TBL) should be repeated within 2 to 4 days to confirm the abnormality and to determine if it is increasing or decreasing, if one or more of these conditions occur:

If a participant with baseline results of…	Developed the following elevations:
ALT or AST <1.5 x ULN	ALT or AST ≥ 5x ULN or ALT or AST ≥ 3x ULN with total bilirubin ≥ 2x ULN
AST or ALT ≥ 1.5 x ULN	ALT or AST ≥ 3x ULN or ALT or AST ≥ 2x ULN with total bilirubin ≥ 2x ULN

If hepatic abnormality persists or worsens, clinical and laboratory monitoring and evaluation for possible causes of abnormal liver tests should be initiated by the investigator. At a minimum, this evaluation should include physical examination as clinically indicated, and a thorough medical history, including symptoms, recent illnesses (for example, heart failure, systemic infection, hypotension, or seizures), history of concomitant medications (including over-the-counter, herbal, and dietary supplements), history of alcohol drinking, and other substance use. In addition, the evaluation should include a blood test for prothrombin time (PT-INR); serological tests for viral hepatitis and autoimmune hepatitis; and an abdominal imaging study (for example, ultrasound or CT scan). Based on the patient's history and initial evaluation results, further testing should be considered, including tests for hepatitis D virus (HDV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), acetaminophen levels, acetaminophen protein adducts, urine toxicology screen, Wilson's disease, blood alcohol levels, urinary ethyl glucuronide, and blood phosphatidyl-ethanol. Based on the circumstances and the investigator's assessment of the participant's clinical condition, the investigator should consider referring the participant for a hepatologist or gastroenterologist consultation, magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), cardiac echocardiogram, and/or a liver biopsy.

Hepatic Monitoring Tests for a Hepatic Treatment Emergent Abnormality

Hematology	**Clinical Chemistry**
Hemoglobin	Total bilirubin
Hematocrit	Direct bilirubin
Erythrocytes (RBCs – red blood cells)	Alkaline phosphatase (ALP)
Leukocytes (WBCs – white blood	Alanine aminotransferase (ALT)
Differential:	Aspartate aminotransferase (AST)

Neutrophils, segmented	Gamma-glutamyl transferase (GGT)
Lymphocytes	Creatine kinase (CK)
Monocytes	**Other Chemistry**
Basophils	Acetaminophen
Eosinophils	Acetaminophen protein adducts
Platelets	Alkaline phosphatase isoenzymes
Cell morphology (RBC and WBC)	Ceruloplasmin
	Copper
Coagulation	Ethyl alcohol (EtOH)
Prothrombin time, INR (PT-INR)	Haptoglobin
	Immunoglobulin IgA (quantitative)
Serology	
Hepatitis A virus (HAV) testing:	Immunoglobulin IgG (quantitative)
HAV total antibody	Immunoglobulin IgM (quantitative)
HAV IgM antibody	Phosphatidylethanol (Peth)
Hepatis B virus (HBV) testing:	**Urine Chemistry**
Hepatitis B surface antigen (HbsAg)	Drug screen
Hepatitis B surface antibody (anti-HBs)	Ethyl glucuronide (EtG)
Hepatitis B core total antibody (anti-HBc)	**Other Serology**
Hepatitis B core IgM antibody	Anti-nuclear antibody (ANA)
Hepatitis B core IgG antibody	Anti-smooth muscle antibody (ASMA) [a]
HBV DNA [c]	Anti-actin antibody [b]
Hepatis C virus (HCV) testing:	Epstein-Barr virus (EBV) testing:
HCV antibody	EBV antibody
HCV RNA [c]	EBV DNA [c]
Hepatitis D virus (HDV) testing:	Cytomegalovirus (CMV) testing:
HDV antibody	CMV antibody
Hepatitis E virus (HEV) testing:	CMV DNA [c]
HEV IgG antibody	Herpes simplex virus (HSV) testing:
HEV IgM antibody	HSV (Type 1 and 2) antibody
HEV RNA [c]	HSV (Type 1 and 2) DNA [c]

	Liver kidney microsomal type 1 (LKM-1) antibody
Microbiology	
Culture:	
Blood	
Urine	

Interstitial lung disease (ILD)/ pneumonitis events

Interstitial lung disease (ILD)/pneumonitis has been identified as an adverse drug reaction for abemaciclib. The majority of events observed in clinical trials were Grade 1 or Grade 2, with serious cases and fatal events reported. Monitor for clinical symptoms such as hypoxia, dyspnea, cough, and fever, and or radiological changes indicative of ILD/pneumonitis. Ask your patients to report any new or worsening pulmonary symptoms and investigate and treat as per your local clinical practice (including corticosteroids, as appropriate). If ILD/pneumonitis is suspected, investigations may include imaging, such as high-resolution computed tomography, bronchioalveolar lavage, and biopsy as clinically indicated.

Discontinue abemaciclib in cases of severe (Grade 3 or 4) ILD/pneumonitis.

CTCAE Grade	Abemaciclib dose modifications
Grade 1 or 2	No dose modification is required.
Persistent or recurrent Grade 2 toxicity that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Suspend dose until toxicity resolves to baseline or Grade 1. Resume at next lower dose.
Grade 3 or 4	Discontinue abemaciclib.

Venous thromboembolic events (VTE)

VTE has been identified as an adverse drug reaction for abemaciclib and other CDK4/6 inhibitors in combination with endocrine therapy. At this time, the mechanism underlying the association between abemaciclib and the occurrence of VTEs is not known, and seems to be higher when abemaciclib is combined with tamoxifen. Monitor patients for signs and symptoms of deep vein thrombosis and pulmonary embolism and treat as medically appropriate. In cases of VTE, patients must suspend abemaciclib, and VTE must be treated as clinically indicated. Abemaciclib may be resumed when the patient is clinically stable. Endocrine treatment can be continued as judged by the investigator.

Increase in serum creatinine and assessment or 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. If there is a concern about elevated creatinine, a cystatin C can be sent to confirm normal

renal function.

Other adverse events not otherwise specified:

CTCAE Grade	Abemaciclib dose modifications
Grade 1 or 2	No dose modification is required.
Persistent or recurrent Grade 2 toxicity that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Suspend dose until toxicity resolves to baseline or Grade 1. Resume at next lower dose.
Grade 3 or 4	Suspend dose until toxicity resolves to baseline or Grade 1. Resume at next lower dose.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Expected Toxicities

7.1.1 Adverse Event List for Abemaciclib

Abemaciclib has an acceptable safety profile in the phase 2 study MONARCH 1 and multiple phase 3 trials (MONARCH 2 and MONARCH 3). The safety monitoring and corresponding dose adjustment guidelines used in these clinical studies effectively improved the tolerability profile of abemaciclib. The most frequently reported adverse events ([AEs], >20%, consistently in these 3 studies) are: diarrhea, neutropenia, fatigue, nausea, vomiting, abdominal pain, decreased appetite, and anemia. Additionally, other clinically relevant AEs are venous thromboembolism (VTE, [including pulmonary embolism (PE) and deep vein thrombosis (DVT)]), ALT increased, AST increased, and interstitial lung disease (ILD)/pneumonitis.

Please see the current Investigator's Brochure for Abemaciclib for complete and up to date safety information.

7.1.2 Adverse Event List for Endocrine Therapy

The most common adverse events experienced with use of AIs include hot flashes, arthralgias, and gradual loss of bone density. The most common adverse events experienced with use of tamoxifen include hot flashes, night sweats, and vaginal discharge. Venous thromboembolic disease and endometrial cancer are rare risks of tamoxifen.

Detailed descriptions of anticipated AEs for the 3 AIs, LHRH agonist, and tamoxifen should be obtained from the package inserts (SmPCs) of the locally obtained commercial supplies.

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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 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.

- **Expectedness of the AE:**
  - **Expected** adverse events are those adverse events that are listed or characterized in the current adverse event list, the Package Insert, the Investigator Brochure or is included in the informed consent document as a potential risk.
  - **Unexpected** adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected.

7.3 Adverse Event Reporting

- In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Sponsor-Investigator.
- Investigators must report to the Sponsor-Investigator any adverse event (AE) 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.
- For Multi-Center Trials where a DF/HCC investigator is serving as the Sponsor-Investigator, each participating institution must abide by the reporting requirements set by the DF/HCC**.** The Sponsor-Investigator and Coordinating Center should be notified of SAEs within 1 working day of learning of the event.

7.4 DF/HCC Expedited Adverse Event Reporting Guidelines

The following adverse events must be reported to the DFCI IRB according to the expedited reporting guidelines:

- All locally occurring Adverse Events that are *Serious*, *Unexpected*, and there is a *Reasonable Possibility* the Adverse Event is related to the study intervention should be reported to the IRB.

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

- All life threatening and fatal Serious Adverse Events (grade 4 and 5) considered serious, unexpected, and related, must be submitted via a written adverse event report to OHRS **within 5 working days** from notification of the event.
- For all other Serious Adverse Events considered serious, unexpected, and related, a full written adverse event report must be submitted to OHRS **within 10 working days** from notification of the event.
- Any unrelated adverse event does not require reporting to OHRS except grade 5 events which must be reported at the time of continuing review.

Other investigative sites will report SAEs to their respective IRB according to their local IRB's policies and procedures for reporting adverse events. A copy of the submitted institutional AE form will be forwarded to the Coordinating Center.

The Coordinating Center will submit AE reports from outside institutions to the DFCI IRB according to DFCI IRB policies and procedures in reporting adverse events.

7.5 Routine Adverse Event Reporting

Grade 2 and higher Adverse Events **must** be reported in routine study data submissions to the PI on the toxicity case report forms. Abnormal laboratory results are only reported as adverse events in the case report forms if deemed to be clinically significant by a treating investigator**. AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

7.6 Expedited Adverse Event Reporting to Eli Lilly

A serious adverse event (SAE) is any adverse event that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment that results in one of the following outcomes:

(1) death
(2) in-patient hospitalization or prolonged hospitalization
(3) life-threatening
(4) persistent or significant disability or incapacity
(5) congenital anomaly or birth defect
(6) other serious events that may jeopardize the patient and may require medical or

surgical intervention to prevent one of the other five listed outcomes.

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require expedited reporting to Eli Lilly unless otherwise specified. The event can be reported as:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined below.

Previously planned (prior to signing the informed consent form) surgeries, and non-disease related elective surgeries planned during the course of the study, should not be reported as SAEs unless the underlying medical condition has worsened or appeared during the course of the study. Events that occur prior to the first administration of study medication should not be reported as SAEs.

Pre-planned hospitalizations or procedures for pre-existing conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs.

Hospitalization or prolongation of hospitalization without a precipitating clinical AE (e.g., for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Death due to disease progression should not be reported as an SAE unless the investigator deems it to be related to the use of study drug.

SAEs will be reported within 15 business days of awareness of "serious" adverse event on the MedWatch 3500A and will be submitted to Eli Lilly via email: [redacted]

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

The Sponsor-Investigator will be responsible for all communications with the FDA. The Sponsor-Investigator 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.

7.8 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant

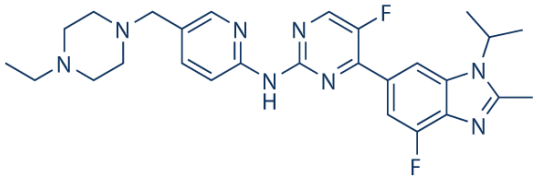
safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

8. PHARMACEUTICAL 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.

8.1 Abemaciclib (LY2835219, Verzenio®)

8.1.1 Description

Formula	**$C_{27}H_{32}F_2N_8$**
Chemical structure	
IUPAC	N-[5-[(4-ethylpiperazin-1-yl)methyl]35yridine-2-yl]-5-fluoro-4-(7-fluoro-2-methyl-3-propan-2-ylbenzimidazol-5-yl)pyrimidin-2-amine
Molecular weight	506.6 g/mol
Physicochemical properties	Practically white to yellow powder
Solubility	-pH dependent solubility: considered highly soluble, with solubility ≥5 mg/mL up to pH 6.0 and 1.577 mg/mL at pH 6.8 in aqueous media -Water: 0.001 mg/mL -Ethanol: 22.313 mg/mL
Mechanism of clearance	– mainly hepatic metabolism – mediated by CYP3A4 – major metabolite: N-desethylabemaciclib (M2) – other metabolites: hydroxyabemaciclib (M20), hydroxy-N-desethylabemaciclib (M18), and an oxidative metabolite (M1). – M2, M18, and M20 are equipotent to abemaciclib – geometric mean hepatic clearance: 26 L/h
Mean plasma T ½	18.3 hours

8.1.2 Form

Abemaciclib will be provided as 50 mg tablets in bottles.

8.1.3 Storage and Stability

Abemaciclib does not require any special storage conditions. The product is stable when stored at room temperature conditions according to the label. Shelf life is 3 years.

8.1.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to

themselves and the environment, should undertake the preparation, handling, and safe disposal of abemaciclib in a self-contained and protective environment.

8.1.5 Availability
Abemaciclib is provided by Eli Lilly and Company from investigational supply for the duration of this clinical trial.

8.1.6 Administration
Abemaciclib tablets should be dispensed in a well-closed, light-resistant container. Abemaciclib is administered orally twice per day, at the total dose of 50 mg, 100 mg or 150 mg based on the dose-escalation schedule in this protocol. Abemaciclib should be taken at the same time every 12 hours ±4 hours with a glass of water. Abemaciclib must be taken with at least 6 hours separating doses. Tablets must be swallowed intact, as provided, and they are not intended to be chewed or crushed. Abemaciclib can be taken with or without food. Doses that would occur outside of this timeframe should be considered missed and should not be administered; participants should be advised that they should never double dose. If a participant vomits following a dose, the dose should not be re-taken; instead, the participant should continue with their next regularly scheduled dose as clinically appropriate.

8.1.7 Ordering
Abemaciclib will be ordered from Eli Lilly and Company by each participating site.

8.1.8 Accountability
The investigator at the participating site, 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.)

8.1.9 Destruction and Return
Expired, returned, or unused supplies of abemaciclib should be destroyed according to local institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 Endocrine Therapy

8.2.1 Description, Form, and Preparation
Locally obtained commercial supply of tamoxifen, anastrozole, letrozole, exemestane, or LHRH agonists will be administered to the patients at the discretion of the principal investigator (or his/her designee) as well as according to standard institutional or regional practice. Generics are allowed, if locally available.

Refer to the approved FDA package inserts for more information regarding each individual agent.

8.2.2 Storage and Stability
As all endocrine therapy agents will be used as commercially-supplied agents, storage will be at the discretion of each local institution pharmacy's standard practices.

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

8.2.4 Availability
All endocrine agents used on trial are commercially available. The cost of all agents will be charged to the patient and/or his/her insurance company since its use is considered standard of care.

8.2.5 Ordering
Ordering will be at the discretion of each local institution pharmacy's standard practices for the endocrine therapy used on this protocol.

8.2.6 Drug Supplies and Accountability
As all endocrine therapy agents will be used as commercially-supplied agents, they will be supplied and the inventory kept according to local institutional pharmacy practices.

8.2.7 Destruction and Return
Unused or expired supplies of endocrine therapy should be destroyed according to institutional policies.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

The following outline provides a brief overview of the biomarker and correlative studies. The correlative objectives of this study are detailed in section 1.5. Additional exploratory studies not listed here may be pursued in the future if deemed relevant to the study. Research specimens will collected to enable present and future research questions to be addressed with innovative technologies and research methodologies, but also to explore the utility and validity of potential future biomarkers or future technological applications that will help clarify multiple determinants of prognosis and risk of disease recurrence, including as it pertains to tolerability of abemaciclib.

Details on specimen collection, processing and analysis can be found in the Operations Manual.

Sample collection kits for research blood and stool samples will be provided to participating sites.

Specimen Collection Table							
Specimen Type	**Collection Timepoint**					**Shipping Condition**	**Ship to**
	Baseline (after consent prior to tx start)[a]	**C2D1**	**C3D1**	**Every 3 Cycles**	**EOT/PD** (whichever occurs first)[d]		
3 10mL Streck Tubes[b]	X	X	X	X	X	Ambient	Translational Hub – Smith Campus
2 8 mL CPT Tubes	X		X		X	Ambient	Translational Hub – Smith Campus
Stool Sample[c]	X	X			X	Ambient	Translational Hub – Smith Campus
Archival tumor tissue (blocks or slides + H&E)	X					Ambient	DFCI Breast Oncology Translational Team

a. Baseline/Pre-Treatment samples to be collected after registration but prior to initiation of therapy for participating sites outside of DF/HCC (may be collected at C1D1 pre-treatment)
b. Streck tubes will be collected at baseline, C2D1, C3D1 and q3 cycles thereafter.
c. Stool samples will be collected at baseline (pre-dose C1D1), between C2D1 and C3D1, and EOT.
d. EOT includes if a patient finishes 2 years on study treatment, experiences disease recurrence while on study, or comes off study treatment for other reasons per Section 5.5.

9.1 Research Blood Sample Collection

Research blood will be collected at the following timepoints:

Baseline (or on C1D1)

- 3 10mL Streck tubes of whole blood
- 2 8mL CPT tubes for PBMCs
- collected **after** patient registration for sites outside of DF/HCC

Cycle 2 Day 1

- 3 10mL Streck tubes of whole blood

Cycle 3 Day 1

- 3 10mL Streck tubes of whole blood
- 2 8mL CPT tubes for PBMCs

Every 3 cycles

- 3 10mL Streck tubes of whole blood

End of Treatment OR Disease Progression, whichever occurs first

- 3 10mL Streck tubes of whole blood
- 2 8mL CPT tubes for PBMCs

Collection supplies for research blood samples are contained in the research sample collection kit. Please see the Operations Manual for detailed instructions for collection, processing, labeling, and shipping of research blood samples.

Of note, every effort will be made to coordinate research blood draws with clinically-indicated blood tests to minimize additional venipuncture. Research coordinators will track participants closely and make every effort to collect blood at required timepoints. However, if research blood draws are missed or fall outside window due unanticipated changes in treatment/ scheduling issues, the sample will be collected at the subject's next clinic appointment, and this will not be considered a violation of the protocol. These cases should be discussed with the coordinating center.

9.2 Archival Tumor Tissue Collection

Archival tumor tissue specimens will be submitted from either the diagnostic biopsy (from time of first diagnosis) or Surgery (for primary breast cancer). Surgical samples are preferred except in patients who have undergone neoadjuvant treatments, in which case diagnostic biopsy tissue is preferred.

Either 1 paraffin block or 15 unstained slides AND an H&E slide 4-5µm thick will be submitted.

Archival tissue is shipped at ambient temperature in conventional paraffin block or slide shipper with the de-identified pathology report and Specimen Requisition Form found in the Operations Manual. The Operations Manual contains additional information on labeling and shipment of archival tissue to DFCI.

9.3 Stool Specimen Collection

Patients will be provided with collection kits for self-collection of stool sample.

Stool will be collected at the following timepoints:

- Baseline (prior to first dose on C1D1)
- Between C2D1 and C3D1
- End of Treatment

A stool questionnaire is included in the kits (and Appendix D). The questionnaire 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.

All kits will be provided to the patients at a clinic visit. Patients will also be provided with a mailer in which to return the sample. All samples will be shipped at ambient temperature to DFCI where they will be processed and stored (see Operations Manual).

9.4 Patient-Reported Outcome (PROs)

The following PRO assessments are required for English speaking patients:

	Timepoint				
Assessment	**Baseline or C1D1 prior to first dose**	**Cycle 2 Day 1** (+/- 2 wks)	**Cycle 3 Day 1** (+/- 2 wks)	**Every 3 Cycles** (+/- 2 wks)	**EOT** (+/- 4 wk)
FACT Breast	X	X	X	X	X
FACT-Endocrine Symptoms	X	X	X	X	X
FACIT- Fatigue	X	X	X	X	X

The PRO assessments should be completed in RedCap via tablet or on any available computer with an internet connection. If the participant cannot complete the assessments electronically, paper versions are available in the questionnaire packet. The site study team is responsible for transcribing the paper completed assessments into the electronic forms in RedCap and retaining the original paper forms as source documentation.

All the FACT/FACIT questionnaires, subscales, and items are scaled using a 5-point Likert rating ranging from O not at all' through 4 'very much'. The recall period is the past 7 days. It usually takes 2 to 3 minutes to complete 10 questions.

Refer to the TRADE Operations Manual for additional instructions.

10. STUDY CALENDAR

Baseline evaluations are to be conducted during the screening window of the TRADE trial, prior to the start of protocol therapy (within 30 days of enrollment). All baseline assessments must be performed prior to administration of abemaciclib. If performed at baseline $\leq$ 7 days of C1D1, procedures do not need to be repeated to initiate treatment.

After the completion of 3 cycles, visits will occur every 3 cycles through 2 years. More frequent visits are acceptable if necessary for closer monitoring of toxicity. Subjects will receive study treatment for 2 years or until meeting another withdrawal criterion. Visits should occur within +/- 7 days of the scheduled day unless otherwise specified, a -7 day window can be used if an assessment is performed in advance of a study visit.

All subjects will be asked to return to the site for a final End-of-Treatment visit. The End-of-Treatment visit must be performed within 30 days of final administration of study treatment. End-of-treatment assessments will not have to be repeated if the same assessments were performed within 7 days of this visit.

Tests and procedures	**Within 30 days prior to enrollment**	**Treatment Phase (2 years total)**						**End Of Treatment / Off-Study Visit**	**EDC Timepoints (To be used by RIO only)**
		Cycle 1 Day 1	Cycle 1 Day 15 (+/- 3 days)	Cycle 2 Day 1	Cycle 2 Day 15[f] (+/- 3 days)	Cycle 3 Day 1	Every 3 cycles after C3D1	within 30 days of last dose	
Demographics	X								Baseline only
Medical History	X								Baseline only
Physical Exam	X	X	X	X		X	X	X	
Vital signs[b]	X	X	X	X		X	X	X	
Height/Weight	X								Baseline only
ECOG Performance status	X	X		X		X	X	X	All timepoints
CBC with differential	X	X	X	X	X	X	X	X	
COMP[c]	X	X	X	X	X	X	X	X	
Serum or urine pregnancy test[a]	X								
Drug Diary Review/ Drug Accountability	X	X	X	X		X	X	X	All timepoints
AE Assessment	X	X	X	X		X	X	X	All timepoints
Con Med Review	X	X	X	X		X	X	X	All timepoints
Archival Tumor Tissue Retrieval[e]	X								
Research Blood[e]	X			X		X	X	X	
Research Stool Sample[e]	X			X				X	
PRO questionnaires[e]	X			X		X	X	X	

*Visits should occur within +/- 7 days of the scheduled day unless otherwise specified.

a. Only required for patients of childbearing potential.
b. Vitals should include: diastolic and systolic blood pressure and heart rate.
c. COMP should include: sodium, potassium, chloride, bicarbonate, BUN, serum creatinine, glucose, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin
d. Tests performed at C3D1 will continue every three cycles (cycle 6, 9, 12 , and so forth) until EOT.
e. See Section 9 for details surrounding archival tissue, research blood, research stool, and PRO questionnaires.
f. Cycle 2 Day 15 bloodwork can be drawn at a local laboratory with results faxed to study site for review by treating team

11. MEASUREMENT OF EFFECT

The primary endpoint of the TRADE study will capture a composite incidence of treatment discontinuations, dose reductions, and/or inability to reach and maintain the full dose of abemaciclib.

11.1 Safety profile, therapeutic adherence, and quality of life

For the purpose of this study, patients will be monitored for treatment emergent adverse events, as well as consequential dose interruptions and need for dose reduction. Also, non-achievement of the target full dose of abemaciclib will be registered.

Report and grading of the adverse events will be based on the Common Terminology Criteria for Adverse Events (CTCAE) version 5.

Therapeutic compliance and treatment intensity is evaluated as:
-Ratio of the self-reported number of pills taken and number of pills prescribed
-Ratio of the cumulative 12-week dose (expressed in mg) of abemaciclib taken and the dose prescribed
-Median daily dose of abemaciclib in weeks 1-2, 2-4, 4-6, and 7-12.
-Rate of patients at full dose (150mg BID) at 12 weeks versus those unable to reach full dose

Quality of life and patient-reported outcomes are evaluated through standardized scales, as assessed in the monarchE pivotal phase 3 trial, for consistent intra-trial comparisons:
-Time to Deterioration of QoL with FACT-B, FACT-ES subscale and FACIT-f

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method
The DF/HCC 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 are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

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 medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Sponsor-Investigator and study team.

The DSMC generally reviews each protocol up to four times a year with the frequency determined by the outcome of previous reviews. 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 across all sites; summary of all deaths occurring within 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 Multicenter Guidelines

This protocol will adhere to DF/HCC Policy MULTI-100 and the requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Sponsor-Investigator, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix B.

12.4 Collaborative Research and Future Use of Data and Biospecimens

Tissue, blood, stool, 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 or 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 anywhere 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

TRADE is an interventional, prospective, single-arm, open label, phase 2 study designed to evaluate tolerability of a dose-escalation strategy of abemaciclib in combination with standard endocrine therapy for the adjuvant treatment of breast cancer. The study intends to analyze a composite endpoint of abemaciclib discontinuation rate, dose modification, and/or inability to reach the full target dose at 12 weeks, compared

with published data from monarchE.[10,12] Enrolled patients will receive an initial dose-escalated modified schedule of abemaciclib in combination with ongoing standard provider-choice endocrine treatment. Patients with early breast cancer who have completed primary surgical, radiotherapy, and chemotherapy treatments (if indicated), and have a medical indication for adjuvant abemaciclib per standard clinical practice, will be potentially eligible. All patients will start abemaciclib at the -2 dose level (50mg BID) for the first 2 weeks of therapy. Patients will proceed to the next dose level only if they do not experience adverse events qualifying for dose-reduction and interruption criteria, or persistent grade 2 or higher toxicity for more than 2 weeks. Following successful completion of the first dose level, patients will increase abemaciclib to -1 dose level level (100 mg BID) for 2 weeks. Patients without toxicity leading to drug hold or reduction will then increase to the final full dose level (150 mg BID as per FDA approved schedule), to be used for the remainder of the planned 2 years of abemaciclib therapy. The primary endpoint will be assessed after 12 weeks of adjuvant abemaciclib therapy. Patients will be followed for their entire course of abemaciclib to determine overall tolerability, completion rates, and compliance.

The primary endpoint of the trial is the Composite Adverse Rate, define as the 12-week rate of abemaciclib discontinuation for any reason and/or abemaciclib dose reduction and/or inability to reach or maintain the target standard dose of abemaciclib.

13.2 Sample Size, Accrual Rate and Study Duration

The pre-test assumptions are based on the trial-based data of monarchE, estimating a rate of abemaciclib discontinuation at 12 weeks of 5% and/or dose reduction of 35% (12-week composite rate: approximately 40%). The experimental hypothesis is that a dose-modified abemaciclib schedule will reduce the rate of the composite primary endpoint at 12 weeks to $\leq$25% from a baseline of 40%. The sample size estimation is based on 92% power (1-β), significant at 1-sided Type I error alpha of 0.07, assuming a drop rate of 10%, to reject the null hypothesis, resulting in a population size of 90 patients. The duration of accrual is anticipated to be approximately 18 months.

If a patient experiences disease recurrence beyond the first 12 weeks of treatment, they are evaluable for the primary endpoint. If they experience recurrence within the first 12 weeks, the assessment of the impact of the dose-escalation schedule is not deemed feasible and the patient will not be included in the primary efficacy analysis. The rate of disease recurrence within 12 weeks is expected to be <5%, based on the monarchE trial.
Subjects who withdraw after signing the informed consent form (ICF) but before receiving study treatment will be considered unevaluable and will be replaced.

13.2.1 Planned Efforts to Reach Accrual Targets

The study team will prioritize ensuring that this trial is presented to all eligible patients, regardless of demographics. The trial will be presented routinely at various clinical research meetings in the breast oncology community within Dana-Farber/Harvard Cancer Center. Additionally, efforts will be made to publicize the trial to patients and providers within the DF/HCC catchment region.

13.2.2 Efforts to Support Retention and Resource Needs of Participants

The study protocol has been developed, and will be implemented, with ongoing input from breast cancer patient advocates regarding patient impacts of the trial requirements. As the budget allows, efforts will be

made to provide parking discounts or vouchers for study participants. In addition, we will make every effort to translate the consent form into relevant languages.

13.2.3 Tracking decline of consent and participant withdrawal
We are not planning to specifically track all patients who have been presented the study and decline consent. We will track the rate of participant withdrawal and reasons for withdrawal after initial signing of written informed consent.

13.3 Analysis of Primary Endpoints

The incidence of the composite endpoint will be reported, including disaggregated and combined rates of treatment discontinuations and/or dose reductions. Data are presented as overall rates and confidence intervals, and significance will be expressed with P-value.

13.4 Analysis of Secondary and Exploratory Endpoints

The incidence of adverse events will be reported as frequencies and absolute numbers, graded based on CTCAE v.5.0.

The incidence of the primary composite endpoint will be reported at 6 and 24 months to evaluate the longer time impact of the dose-escalation strategy.

Additional secondary endpoints to be monitoring throughout the treatment phase include:
- rate of treatment discontinuation
- rate of treatment dose reduction
- rate of inability to reach the full dose (patients who have never reached the full dose at 150mg BID)
- rate of inability to maintain the full dose (patients who have received 150mg BID at least once)
- incidence of clinical meaningful diarrhea (G2-4 CTCAE)

Quality of life and patient-reported outcomes are evaluated through standardized scales, as assessed in monarchE pivotal phase 3 trial, for consistent intra-trial comparisons:
-Time to Deterioration of QoL with FACT-B and FACT-ES subscale, FACIT-f, EQ-5D-5L, and EORTC QLQ-30. Scales are administered at screening, on C1D1, C2D1, C3D1, and EOT.

Dose intensity and therapeutic adherence to abemaciclib will be analyzed and reported with descriptive statistics.

Translational exploratory studies will be performed on histology specimens, ctDNA, and stool samples. The results of these analyses will be reported with descriptive statistics and exploratory correlation with relevant endpoints.

Correlative analyses will be also explored as they pertain to clinical and pathological factors and any impact on survival outcomes.

Sites Performing Correlative Studies and/or Data Analysis
De-identified biospecimens and data may be shared with the following entities for correlative studies:

- Harvard T. H. Chan School of Public Health Microbiome Analysis Core
- Baylor School of Medicine Alrek Center for Metagenomics & Microbiome Research
- Natera Inc

13.5 Reporting and Exclusions

13.5.1 Evaluation of Toxicity
All participants who receive at least one dose of abemaciclib will be evaluable for toxicity.

13.5.2 Evaluation of the Primary Efficacy Endpoint
All eligible participants included in the study who receive at least one dose of study medication will be assessed for the primary composite endpoint, even if there are major protocol therapy deviations.

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.

15. REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2021
2. Siegel RL, Miller KD, Fuchs HE, et al: Cancer statistics, 2022. CA Cancer J Clin 72:7-33, 2022
3. Perou CM, Sorlie T, Eisen MB, et al: Molecular portraits of human breast tumours. Nature 406:747-52, 2000
4. Howlader N, Altekruse SF, Li CI, et al: US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 106, 2014
5. Waks AG, Winer EP: Breast Cancer Treatment: A Review. JAMA 321:288-300, 2019
6. Duggan C, Trapani D, Ilbawi AM, et al: National health system characteristics, breast cancer stage at diagnosis, and breast cancer mortality: a population-based analysis. Lancet Oncol 22:1632-1642, 2021
7. Burstein HJ, Curigliano G, Thurlimann B, et al: Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 32:1216-1235, 2021
8. National Comprehensive Cancer Network: Clinical Practice Guidelines in Oncology. Breast Cancer, Version 4.2022. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf.
9. Andre F, Ismaila N, Allison KH, et al: Biomarkers for Adjuvant Endocrine and Chemotherapy in Early-Stage Breast Cancer: ASCO Guideline Update. J Clin Oncol 40:1816-1837, 2022
10. Harbeck N, Rastogi P, Martin M, et al: Adjuvant Abemaciclib Combined With Endocrine Therapy for High-Risk Early Breast Cancer: Updated Efficacy and Ki-67 Analysis From the monarchE Study. Ann Oncol, 2021
11. Johnston SRD, Toi M, O'Shaughnessy J, et al: Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncol 24:77-90, 2023
12. Rugo HS, O'Shaughnessy J, Boyle F, et al: Adjuvant abemaciclib combined with endocrine therapy for high-risk early breast cancer: safety and patient-reported outcomes from the monarchE study. Ann Oncol 33:616-627, 2022
13. Lynce F, Shajahan-Haq AN, Swain SM: CDK4/6 inhibitors in breast cancer therapy: Current practice and future opportunities. Pharmacol Ther 191:65-73, 2018
14. Dickler MN, Tolaney SM, Rugo HS, et al: MONARCH 1, A Phase II Study of Abemaciclib, a CDK4 and CDK6 Inhibitor, as a Single Agent, in Patients with Refractory HR(+)/HER2(-) Metastatic Breast Cancer. Clin Cancer Res 23:5218-5224, 2017
15. Sledge GW, Jr., Toi M, Neven P, et al: MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. J Clin Oncol 35:2875-2884, 2017
16. Sledge GW, Jr., Toi M, Neven P, et al: The Effect of Abemaciclib Plus Fulvestrant on Overall Survival in Hormone Receptor-Positive, ERBB2-Negative Breast Cancer That Progressed on Endocrine Therapy-MONARCH 2: A Randomized Clinical Trial. JAMA Oncol 6:116-124, 2020
17. Goetz MP, Toi M, Campone M, et al: MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol 35:3638-3646, 2017
18. Johnston S, Martin M, Di Leo A, et al: MONARCH 3 final PFS: a randomized study of abemaciclib as initial therapy for advanced breast cancer. NPJ Breast Cancer 5:5, 2019

19. Johnston SRD, Harbeck N, Hegg R, et al: Abemaciclib Combined With Endocrine Therapy for the Adjuvant Treatment of HR+, HER2-, Node-Positive, High-Risk, Early Breast Cancer (monarchE). J Clin Oncol:JCO2002514, 2020

20. O'Shaughnessy JA, Johnston S, Harbeck N, et al: Abstract GS1-01: Primary outcome analysis of invasive disease-free survival for monarchE: abemaciclib combined with adjuvant endocrine therapy for high risk early breast cancer. Cancer Research 81:GS1-01-GS1-01, 2021

21. Rugo HS, Seneviratne L, Beck JT, et al: Prevention of everolimus-related stomatitis in women with hormone receptor-positive, HER2-negative metastatic breast cancer using dexamethasone mouthwash (SWISH): a single-arm, phase 2 trial. Lancet Oncol 18:654-662, 2017

22. Barcenas CH, Hurvitz SA, Di Palma JA, et al: Improved tolerability of neratinib in patients with HER2-positive early-stage breast cancer: the CONTROL trial. Ann Oncol 31:1223-1230, 2020

23. Martin M, Holmes FA, Ejlertsen B, et al: Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 18:1688-1700, 2017

24. Schmidt M, Lubbe K, Decker T, et al: A multicentre, randomised, double-blind, phase II study to evaluate the tolerability of an induction dose escalation of everolimus in patients with metastatic breast cancer (DESIREE). ESMO Open 7:100601, 2022

25. Ponziani FR, Bhoori S, Germini A, et al: Inducing tolerability of adverse events increases sorafenib exposure and optimizes patient's outcome in advanced hepatocellular carcinoma. Liver Int 36:1033-42, 2016

26. Thibault S, Hu W, Hirakawa B, et al: Intestinal Toxicity in Rats Following Administration of CDK4/6 Inhibitors Is Independent of Primary Pharmacology. Mol Cancer Ther 18:257-266, 2019

27. O'Connell AE, Zhou F, Shah MS, et al: Neonatal-Onset Chronic Diarrhea Caused by Homozygous Nonsense WNT2B Mutations. Am J Hum Genet 103:131-137, 2018

28. Maurer B, Brandstoetter T, Kollmann S, et al: Inducible deletion of CDK4 and CDK6 - deciphering CDK4/6 inhibitor effects in the hematopoietic system. Haematologica 106:2624-2632, 2021

29. Ismail RK, van Breeschoten J, Wouters M, et al: Palbociclib dose reductions and the effect on clinical outcomes in patients with advanced breast cancer. Breast 60:263-271, 2021

30. Zheng J, Yu Y, Durairaj C, et al: Impact of Dose Reduction on Efficacy: Implications of Exposure-Response Analysis of Palbociclib. Target Oncol 16:69-76, 2021

31. Hart LL, Bardia A, Beck JT, et al: Impact of ribociclib (RIB) dose modifications (mod) on overall survival (OS) in patients (pts) with HR+/HER2- advanced breast cancer (ABC) in MONALEESA(ML)-2. Journal of Clinical Oncology 40:1017-1017, 2022

32. De Laurentiis M, Merino LdlC, Hart L, et al: 331P Impact of ribociclib (RIB) dose reduction on overall survival (OS) in patients (pts) with HR+/HER2− advanced breast cancer (ABC) in MONALEESA (ML) -3 and -7. Annals of Oncology 31:S378-S379, 2020

33. Kristensen KB, Thomsen IMN, Berg T, et al: Dose modifications of ribociclib and endocrine therapy for treatment of ER+ HER2- metastatic breast cancer. Breast Cancer Res Treat 188:799-809, 2021

34. Fernandez-Cuerva C, Chinchilla-Alarcon T, Alcaraz-Sanchez JJ: Real-world effectiveness of ribociclib in metastatic breast cancer patients: Does dose affect survival? J Oncol Pharm Pract:10781552221144280, 2022

35. Burris HA, Chan A, Bardia A, et al: Safety and impact of dose reductions on efficacy in the randomised MONALEESA-2, -3 and -7 trials in hormone receptor-positive, HER2-negative advanced breast cancer. Br J Cancer 125:679-686, 2021

36. Schaer DA, Beckmann RP, Dempsey JA, et al: The CDK4/6 Inhibitor Abemaciclib Induces a T Cell Inflamed Tumor Microenvironment and Enhances the Efficacy of PD-L1 Checkpoint Blockade. Cell

Rep 22:2978-2994, 2018

37. Wu Y, Shrestha P, Heape NM, et al: CDK4/6 inhibitors sensitize gammaherpesvirus-infected tumor cells to T-cell killing by enhancing expression of immune surface molecules. J Transl Med 20:217, 2022

38. Charles A, Bourne CM, Korontsvit T, et al: Low-dose CDK4/6 inhibitors induce presentation of pathway specific MHC ligands as potential targets for cancer immunotherapy. Oncoimmunology 10:1916243, 2021

39. Heckler M, Ali LR, Clancy-Thompson E, et al: Inhibition of CDK4/6 Promotes CD8 T-cell Memory Formation. Cancer Discov 11:2564-2581, 2021

40. Li Y, Dong B, Wu W, et al: Metagenomic Analyses Reveal Distinct Gut Microbiota Signature for Predicting the Neoadjuvant Chemotherapy Responsiveness in Breast Cancer Patients. Front Oncol 12:865121, 2022

16. 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.

17. APPENDIX B DF/HCC MULTICENTER DATA & SAFETY MONITORING PLAN

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1. INTRODUCTION

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

1.1 Purpose

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

2 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the following general responsibilities apply, in addition to those outlined in DF/HCC Policies:

2.1 Coordinating Center

The Coordinating Center is the entity that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines).

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

- Assist in protocol development.
- Maintain FDA correspondence, as applicable.
- Review registration materials for eligibility and register participants from External Sites in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to External Sites as needed.
- Review and approve External Site informed consent forms
- Oversee the data collection process from External Sites.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by External Sites and provide to the DF/HCC Sponsor for timely review and submission to the IRB of record, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the reporting requirements for the IRB of record to all External Sites.
- Provide External Sites with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Sites either by on-site or remote monitoring.
- Maintain Regulatory documents of all External Sites which includes but is not limited to the following: local IRB approvals/notifications from all External Sites, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites

- Conduct regular communications with all External Sites (conference calls, emails, etc) and maintain documentation all relevant communications.

2.2 External Site

An External Site is an institution that is outside the DF/HCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC investigator. The External Site acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Each External Site is expected to comply with all applicable DF/HCC requirements stated within this Data and Safety Monitoring Plan and/or the protocol document.

The general responsibilities for each External Site may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their IRB of record.
- Maintain regulatory files as per ICH GCP and federal requirements.
- Provide the Coordinating Center with regulatory documents, study logs (e.g., screening and enrollment, minor violation, biospecimen, etc.) or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required.
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities when required by the sponsor.
- Submit Serious Adverse Event (SAE) reports to sponsor, Coordinating Center, and IRB of record as applicable, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to the Sponsor, Coordinating Center, and IRB of record as applicable, in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.
- Notify the sponsor immediately of any regulatory authority inspection of this protocol at the External Site.

3 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

Certain DF/HCC Policy requirements apply to External Sites participating in DF/HCC research. The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Revisions and Closures

The External Sites will receive notification of protocol revisions and closures from the Coordinating Center. When under a separate IRB, it is the individual External Site's responsibility to notify its IRB of these revisions.

- **Protocol revisions:** External Sites will receive written notification of protocol revisions from the Coordinating Center. All protocol revisions should be IRB approved and implemented within a timely manner from receipt of the notification.

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

3.2 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for External Sites. The External Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each External Site upon request.

External Sites must send their version of the informed consent document to the Coordinating Center for sponsor review and approval prior to local submission. If the HIPAA authorization is a separate document, please submit to the sponsor for the study record. Once sponsor approval is obtained, the External site may submit to their IRB of record, as applicable. In these cases, the approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each External Site will identify the appropriate members of the study team who will be obtaining consent and signing the consent form for protocols. External Sites must follow the DF/HCC requirement that for all interventional drug, biologic, or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

3.3 IRB Re-Approval

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

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

3.4 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended

that the assigned protocol case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.5 Participant Registration

Please refer to Protocol Section 4.3 and 4.4 for participant registration information. Treatment cannot begin until site has received confirmation that participant has been registered with DF/HCC CTMS.

3.5.1 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the External Site receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and IRB of record must be notified of any violations to this policy.

3.5.2 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without IRB of record approval will be permitted. All External Sites are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.6 Data Management

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

Data should be entered promptly and completely after each assessment, but no later than 2 weeks after the completion of a study visit/timepoint.

3.6.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

If study forms are not submitted on schedule, the External Sites will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

3.7 Protocol Reporting Requirements

3.7.1 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure

that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor and to the IRB of record.

3.7.2 Reporting Procedures

Requests to deviate from the protocol require approval from the IRB of record, the sponsor and the IRB of record, where applicable.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

3.7.3 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements, and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

4 MONITORING: QUALITY CONTROL

The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

4.1 Ongoing Monitoring of Protocol Compliance

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

The Coordinating Center will implement ongoing monitoring activities to ensure that External Sites are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

Additionally, a plan will be formulated to provide regular and ongoing communication to External Sites about study related information which will include participation in regular teleconferences initiated by the Coordinating Center. Teleconferences will occur monthly and will continue regularly until completion of protocol therapy. Upon completion of protocol therapy, teleconferences will occur every 3 months until study completion. Additional communication may be distributed via “Newsletter” or email as deemed appropriate by DF/HCC Sponsor.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. External Sites will be required to provide access to participants’ complete medical record and source documents for source documentation

verification during the visit. In addition, External Sites should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the External Site. On-site monitoring visits can be substituted with remote (virtual) monitoring visits at the discretion of the Principal Investigator.

Remote Monitoring: Remote monitoring will be performed on an as-needed basis by the Clinical Trial Monitor. Sites will be asked to provide source documentation via fax, email, or dropbox as specified by the Clinical Trial Monitor for virtual monitoring.

4.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at External Sites that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

4.3 Accrual Monitoring

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

5 AUDITING: QUALITY ASSURANCE

5.1 DF/HCC Internal Audits

All External Sites are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

5.2 Audit Notifications

It is the External Site's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

5.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the IRB of record as applicable.

5.4 External Site Performance

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

External Sites that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be put on hold or closed.

18. APPENDIX C INFORMATION ON POSSIBLE DRUG INTERACTIONS

The information below is provided as guidance to investigators, but is not an exhaustive list of CYP3A4 inducers or inhibitors. Please refer to Section 5.4 for concomitant medication guidelines.

Pharmacological interactions with abemaciclib.

- **CYP3A4 strong inducers**: carbamazepine, dexamethasone (supportive care is allowed ≤7days preferably), phenobarbital, phenytoin, rifapentine, rifampin, rifabutin, St John's wort.
- **CYP3A4 strong inhibitors**: HIV protease inhibitors, clarithromycin, itraconazole, ketoconazole, nefazodone.

Letrozole, anastrozole and exemestane: no known major pharmacological interactions of clinical relevance.

19. APPENDIX D STOOL QUESTIONNAIRE

Breast Cancer Stool Collection Questionnaire

Subject ID:	Date collected _____/_____/_____ Month Day Year	Hour collected _____ ○ am ○ pm

1. Based on the small chart included in the postcard, what did the stool you put into the tube look like? (Choose one or two answers).

☐	Type 1 	Separate hard lumps, like nuts (hard to pass)
☐	Type 2 	Sausage-shaped but lumpy
☐	Type 3 	Like a sausage but with cracks on the surface
☐	Type 4 	Like a sausage or snake, smooth and soft
☐	Type 5 	Soft blobs with clear-cut edges
☐	Type 6 	Fluffy pieces with ragged edges, a mushy stool
☐	Type 7 	Watery, no solid pieces. Entirely Liquid

2. Prior to this collection, when was your last bowel movement?

Earlier today, in the last 6 hours	Earlier today, more than 6 hours ago	Yesterday	Two days ago	More than two days ago
☐	☐	☐	☐	☐

3. In the past 2 months, how often have you had a bowel movement and what was the frequency of stool with the following textures?

	More than twice per day	Twice per day	Once per day	Every other day	Every 3-6 days	Once a week or less	Never
Any bowel movement	☐	☐	☐	☐	☐	☐	
Stool texture (The answers can be different from the above)							
Hard / lumpy	☐	☐	☐	☐	☐	☐	☐
Soft / smooth	☐	☐	☐	☐	☐	☐	☐
Watery liquid	☐	☐	☐	☐	☐	☐	☐

4. In the past 2 months, have you used any of the following medications?

	No	Yes, occasionally	Yes, regularly
Oral antibiotics	☐	☐	☐
If yes, what is the name (list of used)?			
Injected antibiotics	☐	☐	☐
If yes, what is the name (list of used)?			
Prilosec, Nexium, Prevacid (Iansoprazole), Protonix, Aciphex	☐	☐	☐
H2 blocker: Pepcid, Tagamet, Zantac, Axid	☐	☐	☐

5. In the past 2 months, have you used any medications modifying bile production, including (but not limited to): Cholesytramine (e.g. Questran, Prevalite, Locholest), colestipol (e.g, colestid), colesevelam (e.g. Welchol), chenodeoxycholic acids (e.g. CDCA), or ursodeoxycholic acid (e.g. UDCA, Ursodiol, Actigall)?

☐No ☐Yes, occasionally ☐Yes, regularly

6. In the past 2 months, have you undergone a colonoscopy or other procedure requiring bowel preparation? ☐No ☐Yes

7. In the past week, have you taken (or eaten) any of the following? If so, please specify the frequency.

	Not used	Once or twice	Three to six times	Daily	More than once per day
Fiber substitute, such as Metamucil, Konsyl or Citracel	☐	☐	☐	☐	☐
Laxatives, such as Ex-lax, Dulcolax, MiraLax, Senna, or enema	☐	☐	☐	☐	☐
Stool softener, such as Colace	☐	☐	☐	☐	☐
Probiotic supplements	☐	☐	☐	☐	☐
Yogurt or kefir	☐	☐	☐	☐	☐
Other fermented foods, such as sauerkraut or kombucha	☐	☐	☐	☐	☐

8. In the past 6 months, did you gain or lose weight?

No	Gained ≤5 lbs.	Gained >5 lbs.	Lost ≤5 lbs.	Lost >5 lbs.
☐	☐	☐	☐	☐

9. Did you have any problems or concerns with the stool sample collection, for example the solution spilled out of the tube or you had problems with catching stool in the toilet accessory? (Please describe)

_____

_____

_____

Diarrhea Action Plan

You may experience diarrhea while taking your medication. You should treat it as soon as you first notice symptoms.
Make sure you have anti-diarrheal medication on hand.

If you experience loose stools:

- **Start anti-diarrheal medication (Loperamide) as soon as possible** *and follow your clinician's instructions:*
  _____
  _____
  _____
- **Contact your healthcare provider that day.**
- Continue your medication(s) until your healthcare provider tells you to stop.
- Call your healthcare provider again if you still have diarrhea after 24 hours.

Phone number of healthcare provider: _____

Recommendations for eating and drinking:

- ✓ **at least 8 glasses of clear fluids per day**
- ✓ small, more frequent meals
- ✓ foods that are easy to digest
- ✓ soft, bland foods
- ✓ sodium and potassium rich food and drinks (bananas, applesauce, oranges, spinach, sweet potatoes without the skin)

Avoid food & drinks that can irritate you or cause gas, for example:

- x milk & milk products
- x sorbitol (sweetener)
- x caffeine or alcohol
- x very hot or cold food or drinks
- x fatty or spicy food
- x fiber-rich foods (wholegrain, bran products, high-fiber fruit and vegetables, nuts & popcorn)