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Window Trial of Abemaciclib for Surgically Resectable, Chemotherapy-Resistant, Triple Negative Breast Cancer (a BEAUTY Study*)

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*Breast Cancer Genome-Guided Therapy Study

MAYO CLINIC
Cancer Center

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Window Trial of Abemaciclib for Surgically Resectable, Chemotherapy Resistant Triple Negative Breast Cancer

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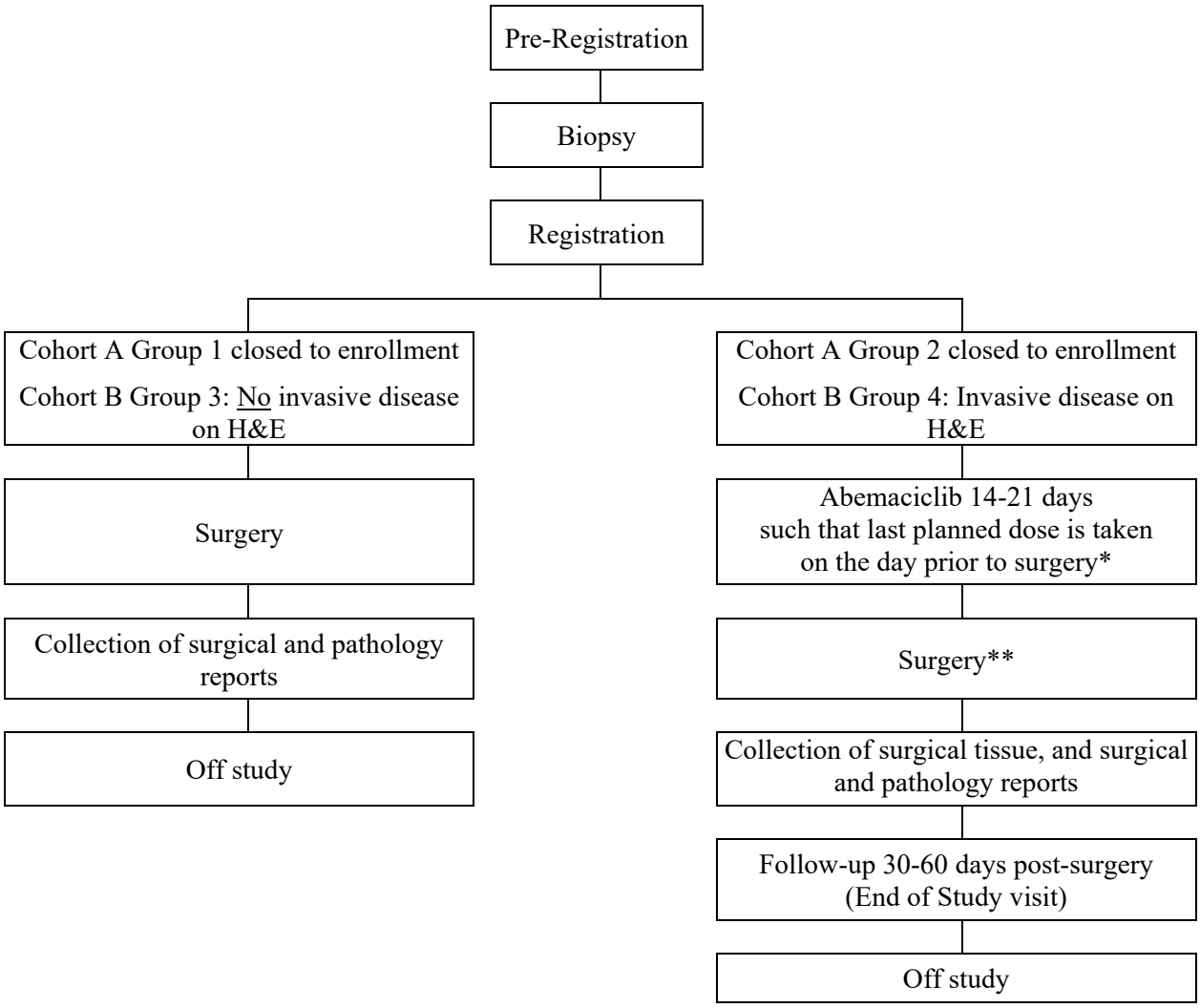
SCHEMA

COHORT A: Patients treated with a neoadjuvant chemotherapy regimen **without** pembrolizumab – Closed with MCCC Amendment 6

COHORT B: Patients treated with a neoadjuvant chemotherapy regimen **in combination with** pembrolizumab



* If abemaciclib is administered for 14-21 days (or up to 42 total doses) and surgery is performed more than 3 days after the last dose of abemaciclib surgical tissue should still be submitted
**Or research biopsy if surgery will not be done

Cycle = 14-21 days of treatment (up to 42 total doses);

NOTE: Patient will not be allowed to register if >56 days after completion of neoadjuvant therapy.

Generic name: abemaciclib Brand name(s): Verzenio® Availability: Eli Lilly & Co supplied

1. STUDY DESIGN/SUMMARY

Triple negative breast cancer (TNBC), defined by a lack of estrogen receptor (ER) alpha and progesterone receptor (PR) expression and absence of HER2 amplification, is an aggressive and highly metastatic subset. Although neoadjuvant chemotherapy combined with pembrolizumab reduces recurrence and death [ref: PMID: 32101663], chemotherapy resistance remains a major problem. Patients with residual disease after neoadjuvant-based chemotherapy (NAC) have a distant recurrence risk approaching 50%.[1]

At least four well-characterized molecular subtypes of TNBC exist, including basal, mesenchymal, immune-enriched and luminal androgen receptor (LAR) subtypes.[2] These subtypes respond differently to NAC. For example, a higher proportion of women with the LAR subset of TNBC are resistant to NAC and exhibit residual disease compared to non-LAR (e.g. basal) subsets.[3,4] However, it is not known whether the survival benefit of chemotherapy differs amongst these TNBC subsets.

Palbociclib, ribociclib and abemaciclib are cyclin dependent kinase 4 and 6 inhibitors (CDK 4/6i) that have recently been FDA approved for the treatment of ER positive, metastatic breast cancer (MBC), either alone (abemaciclib)[5] or in combination with endocrine therapy (palbociclib,[6,7] ribociclib,[8] and abemaciclib).[9,10] Estrogen receptor (ER) and Rb status are thought to be critical determinants of CDK4/6i antitumor activity.[11]

Recently, there have been a series of pre-clinical and clinical publications documenting novel aspects by which CDK 4/6i promote anti-tumor activity, including in TNBC:

1) Activation of the immune system: Goel et al. identified that CDK 4/6 inhibition promotes antitumor immunity through activation of tumor cell expression of endogenous retroviral elements and intracellular levels of double-stranded RNA, resulting in the production of type III interferons and tumor antigen presentation.[12] Additionally, Goel et al demonstrated that CDK4/6i suppress the proliferation of regulatory T cells and DNA methyltransferase 1, resulting in cytotoxic T-cell-mediated clearance of tumor cells. In this study, using the *MMTV-rtTA/tetO-HER2* tumour model, the combination of abemaciclib plus an anti-PDL1 antibody regressed tumors to a greater degree than either abemaciclib alone or anti-PDL1 alonethe combination of abemaciclib plus an anti-PDL1 antibody regressed to a greater degree than either abemaciclib alone or anti-PDL1 alone. This study demonstrated that the CDK4/6 inhibitor abemaciclib may enhance anti-tumour immunity by increasing the functional capacity of tumour cells to present antigen as well as by reducing the immunosuppressive TReg population.

   Based on the preclinical data outlined above, a phase I study was performed, combining abemaciclib plus pembrolizumab plus anastrozole in post-menopausal HR+, HER2- pts with locally advanced or mBC. Patients received 150-mg abemaciclib orally every 12 hours plus pembrolizumab 200 mg intravenously on Day 1 every 21 days plus anastrozole 1 mg orally every 24 hours. Grade 3/4 AEs included increased alanine aminotransferase and neutropenia (8 pts each, 31%) and increased aspartate aminotransferase (6 pts, 23%) and 2 Grade 5 events related to pneumonitis. Preliminary response assessment demonstrated that 5 patients had a confirmed partial response (19% ORR) and clinical benefit rate (CR+PR+SD persisting for ≥6 months) was 27%.

Therefore, despite strong preclinical rationale, further development of the combination of abemaciclib and pembrolizumab is on hold based on these toxicity data.

2) Effects on epithelial-mesenchymal transition and inhibition of metastases. Epithelial-mesenchymal transition (EMT) is a highly-conserved cellular program in which polarized, immobile epithelial cells are converted to migratory mesenchymal cells, a critical process that occurs during embryogenesis,[13,14] and important for tissue regeneration, tumor progression, metastasis, and chemoresistance.[14-22] Liu et al[23] identified a novel mechanism by which CDK4/6i alter EMT and inhibit metastases in TNBC. They demonstrated that overexpression of DUB3 increased SNAIL levels and conversely, knockdown of DUB3 decreased SNAIL and vimentin protein levels, without affecting mRNA levels. CDK4/6 phosphorylates DUB3 at Ser41, thus activating DUB3. Treatment of cells with palbociclib inhibited DUB3 activity, decreased SNAIL and vimentin expression and decreased cell migration. These findings were confirmed with the drug abemaciclib.

For patients with TNBC treated with NAC with/without pembrolizumab and with residual disease, our specific hypotheses are that abemaciclib treatment for 14-21 days will:

1) Increase CD8 T cells and decrease FOXP3 regulatory cells (i.e., increase CD8/FOX3 ratio)
2) Decrease vimentin expression
3) Increase expression of antigen presentation-associated genes in tumors with functional Rb
4) Inhibit proliferation (Ki-67) within subsets with functional Rb (e.g., LAR)

2. OBJECTIVES

2.1 Primary Objective

(1) To examine the effects of abemaciclib on the CD8/FOXP3 ratio in chemotherapy resistant TNBC patients following a neoadjuvant chemotherapy regimen without the addition of pembrolizumab (Cohort A)

(2) To examine the effects of abemaciclib on the CD8/FOXP3 ratio in chemotherapy resistant TNBC patients following a neoadjuvant chemotherapy regimen with the addition of pembrolizumab (Cohort B)

CD8/FOXP3 protein expression will be determined in tumor tissue obtained by image guided percutaneous breast biopsy after completion of NAC+/- pembrolizumab (prior to abemaciclib) and in residual invasive breast cancer obtained at surgery (after 14-21 days of abemaciclib).

2.2 Secondary Objectives for Cohort A and Cohort B independently

1) Assess abemaciclib toxicities
2) To examine the effects of abemaciclib on the percentage of vimentin expressing invasive cancer cells
3) Within TNBC molecular subtypes (basal, mesenchymal, and LAR), to evaluate the effects of abemaciclib on:

a) the individual elements of tumor grade (mitoses, nuclear pleomorphism, and tubule formation)
b) tumor proliferation (as measured by tumor Ki-67 and serum TKI)
c) pDUB3 as well as EMT markers including SNAIL/SLUG, TWIST, and E-Cadherin as measured by IHC
d) Quantification of tumor-infiltrating lymphocytes (as examined by H&E)

2.3 Exploratory Objectives for Cohort A and Cohort B independently

1) To evaluate the effect of abemaciclib on tumor RNA expression.
2) To evaluate the effects of abemaciclib on the immune phenotype of peripheral blood mononuclear cells (PBMC), by evaluating expression of a panel of cell surface markers optimized of identification of human immune cell subpopulations. This goal will be accomplished using multiplexed single-cell proteomic technologies (e.g. Helios CyTOF platform).
3) To evaluate the effects of abemaciclib on tumor-infiltrating immune cells in formalin-fixed paraffin-embedded (FFPE) tumor sections, using multiplexed imaging technologies (e.g imaging mass cytometry , Nanostring DSP or CODEX) which will include:
   a) genes directly involved in tumor cell antigen presentation (e.g. B2M, HLA-A, HLA-B, HLA-C, TAP1, TAP2, TAPBP)
   b) interferon-stimulated genes (ISGs) that regulate antigen presentation (e.g. STAT1, NLRC5) and other ISGs (e.g. IRFs, OAS2)
   c) genes involved in dsRNA response (e.g. DDX58, DHX58)
   d) genes encoding interferons, including type 3 IFNs (e.g. IFNL1, IFNL2, IFNL3)
   e) genes indicating a cytotoxic T cell response (e.g. PRF1, GZMB)
   f) Treg-specific transcription factor genes (e.g. FOXP3, IKZF2)
4) To assess the difference in the frequency of JAK-2 amplification among patients whose post-abemaciclib CD8/FOXP3 ratio ≥1.6 and that among patients whose post-abemaciclib CD8/FOXP3 ratio <1.6.
5) To generate organoids for future research.
6) To evaluate changes in the microbiome with exposure to abemaciclib.

3. BACKGROUND

3.1 Triple Negative Breast Cancer

Triple negative breast cancer (TNBC), defined by a lack of ERα and PR expression and absence of HER2 amplification, accounts for up to 20% of all breast cancers. This subtype is clinically unique due to its aggressive and metastatic nature, typically presenting in younger women at an advanced stage. Despite extensive efforts to characterize the molecular drivers associated with TNBC, including large scale genomic and proteogenomic sequencing efforts[24-26], standard treatment recommendations (surgery, chemotherapy and radiation) have not changed substantially over the past 20 years.

Systemic chemotherapy is administered in the adjuvant setting (after definitive breast surgery) or neoadjuvant setting, with multiple studies demonstrating that chemotherapy

reduces recurrence and prolongs survival.[27] Chemotherapy is standardly administered in the neoadjuvant setting (prior to surgery) for TNBC. A substantial benefit of this approach is the prognostic information gained from pathological assessment of the tumor bed and lymph nodes after surgery, reflecting the response/resistance to neoadjuvant chemotherapy (NAC). Complete eradiation of invasive cancer within the breast and nodes, called pathological complete response (pCR), is a strong prognostic factor.[1] Although anthracycline and taxane based chemotherapy substantially reduces the risk of recurrence and death, chemotherapy resistance is a major problem. Patients with residual disease after neoadjuvant chemotherapy (no pCR) have a distant recurrence risk approaching 50.[1] **For these reasons, novel therapeutic options, including new biomarkers and drug targets, are greatly needed for TNBC patients with resistant disease.**

3.2 TNBC Subtypes

At least four well characterized molecular subtypes of TNBC exist, including basal, mesenchymal, immune-enriched and LAR subtypes.[2] These subtypes respond differently to NAC. For example, a higher proportion of women with the LAR subset of TNBC treated with NAC exhibit residual disease compared to non-LAR (e.g. basal) subsets.[3,4] However, it is not known whether the survival benefit of chemotherapy differs amongst these TNBC subsets.

Palbociclib, ribociclib and abemaciclib are cyclin dependent kinase 4 and 6 inhibitors (CDK 4/6i) are FDA approved for the treatment of ER positive, metastatic breast cancer (MBC), either alone (abemaciclib)[5] or in combination with endocrine therapy (palbociclib,[6,7] ribociclib,[8] and abemaciclib[9,10]). Estrogen receptor (ER) and Rb status are thought to be critical determinants of CDK4/6i antitumor activity.[11]

3.3 Preclinical activity of CDK 4/6 inhibitors in ER negative breast cancer

Recently, there have been a series of pre-clinical and clinical publications documenting novel aspects by which CDK 4/6i promote anti-tumor activity, including in TNBC:

3.3.1 Activation of the immune system

Goel et al. identified that CDK 4/6 inhibition promotes antitumor immunity through activation of tumor cell expression of endogenous retroviral elements and intracellular levels of double-stranded RNA, resulting in the production of type III interferons and tumor antigen presentation.[12] Additionally, Goel et al demonstrated that CDK4/6i suppress the proliferation of regulatory T cells and DNA methyltransferase 1, resulting in cytotoxic T-cell-mediated clearance of tumor cells.

Goel et al demonstrated that in tumor free mice, both abemaciclib and palbociclib significantly reduced Treg numbers and the Treg:CD8 ratio in the spleen and lymph nodes, demonstrating tumor-independent effects of these agents. The selective suppression of Treg proliferation (and not that of CD8+ or naïve CD4+ T cells) may relate to the fact that Tregs express higher levels of Rb1, a key mediator of CDK4/6 pathway modulation. Notably, Rb1 expression is over 3-fold higher in Tregs than CD8+ T cells.

To determine whether cytotoxic T lymphocytes (CTLs) were involved in abemaciclib-mediated therapeutic efficacy, Goel et al implanted MMTV-rtTA/tetO-HER2 tumor fragments orthotopically into athymic *Foxn1^{nu}* mice maintained on doxycycline, and treated them once tumors were established. In contrast to tumors in immunocompetent mice, abemaciclib-treated tumors in nude mice continued to grow. Furthermore, in tumor-bearing MMTV-rtTA/tetO-HER2 mice treated with an anti-CD8 antibody prior to abemaciclib administration, tumor regression (observed in control IgG treated mice), was significantly mitigated by CD8 neutralization. Hence, tumor regression mediated by CDK4/6 inhibition is, at least in part, dependent on the presence of CTLs.

Finally, using the *MMTV-rtTA/tetO-HER2* tumour model, Goel et al demonstrated that the combination of abemaciclib plus an anti-PDL1 antibody regressed tumors to a greater degree than either abemaciclib alone or anti-PDL1 alone.

Following the publication of Goel et al, others have validated these findings and observed a robust effect of abemaciclib on antigen presentation pathway in Rb-competent breast cancer cells, which are further potentiated in vivo, leading to changes in major histocompatibility complex (MHC) class I and II expression on tumor cells, and antigen-presenting cells. Specifically, Schaer et al demonstrated that abemaciclib treatment modulates antigen presentation mechanisms (e.g., upregulation of MHC class I genes HLA-A, HLA-C, HLA-G, transporter associated with antigen processing TAP-1, immunoproteasome subunit LMP-7 encoded by PSMB8) in tumor cells in vitro, triggering an immune response and “activation” of cytotoxic CD8+ T cells.[28]

Based on these preclinical data, a phase I study was performed, combining abemaciclib plus pembrolizumab plus anastrozole in post-menopausal HR+, HER2- pts with locally advanced or mBC. Patients received 150-mg abemaciclib orally every 12 hours plus pembrolizumab 200 mg intravenously on Day 1 every 21 days plus anastrozole 1 mg orally every 24 hours. Grade 3/4 AEs included increased alanine aminotransferase and neutropenia (8 pts each, 31%) and increased aspartate aminotransferase (6 pts, 23%) and two Grade 5 events related to pneumonitis. Preliminary response assessment demonstrated that 5 patients had a confirmed partial response (19% ORR) and clinical benefit rate (CR+PR+SD persisting for ≥6 months) was 27%. Therefore, despite strong preclinical rationale further development of the combination of abemaciclib and pembrolizumab is on hold based on these toxicity data.

3.3.2 Effects on epithelial-mesenchymal transition and inhibition of metastases

Epithelial-mesenchymal transition (EMT) is a highly-conserved cellular program in which polarized, immobile epithelial cells are converted to migratory mesenchymal cells, a critical process that occurs during embryogenesis,[14,15] and important for tissue regeneration, tumor progression, metastasis, and chemoresistance.[15-22,29] Loss of cell adhesion molecule E-cadherin is a functional event of EMT, altering the phenotype of epithelial cells from noninvasive to invasive.[18,30] SNAIL directly represses CDH, the gene encoding E-cadherin, and activates the expression of invasion-associated genes, thereby promoting EMT.[23,31,32]

Liu et al[23] identified a novel mechanism by which CDK4/6i alter EMT and inhibit metastases in TNBC. They demonstrated that overexpression of DUB3 increased SNAIL levels and conversely, knockdown of DUB3 decreased SNAIL and vimentin protein levels, without affecting mRNA levels. While DUB3 had no apparent effect on cell growth, knockdown of DUB3 greatly inhibited the migratory ability and invasiveness of MDA-MB231 cells and suppressed lung colonization using an experiment MDA-MB-231 metastases model. Liu et al. went on to show that CDK4/6 phosphorylates DUB3 at Ser41, thus activating DUB3. Treatment of cells with palbociclib inhibited DUB3 activity, decreased SNAIL and vimentin expression and decreased cell migration. These findings were confirmed with the drug abemaciclib. In a highly metastatic TNBC patient-derived xenograft (PDX) model derived from the Mayo BEAUTY study, the administration of palbociclib did not alter primary tumor growth but significantly reduced lung and liver metastases. The findings of Liu et al were additionally validated in a companion paper published at the same time by Wu et al.[33] Additionally, in a separate laboratory, Qin et al demonstrated that the EMT markers, vimentin and Snail, were down-regulated with palbociclib treatment in the MDA-MB231 model.[34]

While current SNAIL antibodies are not robust for clinical application, taken together, these data identify a potential new CDK4/6i drug target and suggest that changes in expression of EMT targets such as SNAIL and vimentin be predictive of CDK4/6i drug effect.

3.3.3 Effects on Tumor Grade

Regarding the phenotype of tumor grade, Martin et al recently presented the results of the NeoMonarch clinical study evaluating neoadjuvant abemaciclib alone or in combination with aromatase inhibitors for early stage ER+/HER2- breast cancer.[33] In a blinded analysis, abemaciclib monotherapy for 2 weeks reduced tumor grade in 13/46 patients (28%) compared to anastrozole alone, which reduced tumor grade in 5/49 patients (10%). Similar observations with regard to a change in tumor histological phenotype have been observed in vitro and in vivo. Specifically Goetz et al demonstrated that short term abemaciclib (5 days) altered organoid formation in MDA 436 cells (TNBC, claudin low) while Goel et al demonstrated that prolonged exposure to abemaciclib in vivo (MMTV ER+/HER2+ transgenic tumor) resulted in a more differentiated phenotype (Figure 1).

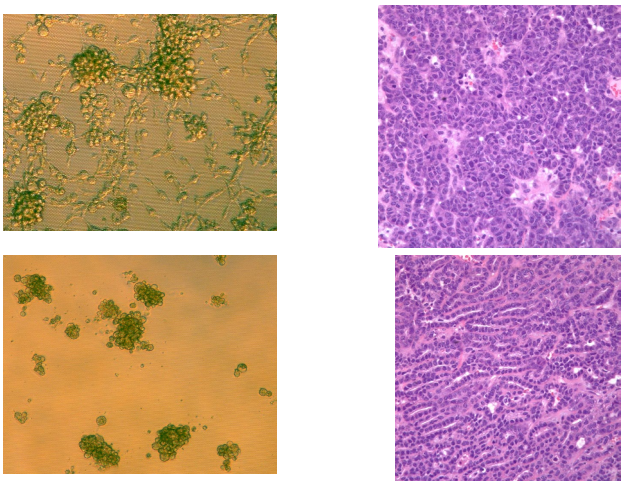


Figure 1. Unpublished data regarding the effects of abemaciclib on 5-day organoid formation (MDA 436)(Goetz) and after prolonged *in vivo* abemaciclib treatment (*MMTV-rtTA/tetO-HER2* transgenic tumors) (Goel)

3.3.4 Effects of CDK4/6 inhibitors in LAR expressing TNBC

Finally, Asghar et al. demonstrated that the luminal androgen receptor (LAR) subtype of TNBC was highly sensitive to CDK4/6 inhibition,[35] potentially extending the use of CDK 4/6i to the LAR subtype, a TNBC subset that is considered resistant to chemotherapy.

3.4 Clinical importance of immune and EMT biomarkers in TNBC.

Miyashita et al demonstrated that following completion of NAC for TNBC, >50% of tumors had a CD8/FOXP3 ratio of 1.6 or higher or a fold change of 1.8 in the CD8/FOXP3 ratio (that is, ratio of post-NAC CD8/FOXP3 ratio value to pre-NAC CD8/FOXP3 ratio).[36] In these patients, recurrence-free survival (RFS) and breast-cancer specific survival (BCSS) were significantly improved for those with post-NAC CD8/FOXP3 ratio of 1.6 or higher or a fold change of 1.8 or higher in the CD8/FOXP3 ratio. Based on preliminary data from the laboratory of Goel et al demonstrating abemaciclib induced suppression of FOXP3 Tregs, our hypothesis is that abemaciclib will suppress Tregs and thus increase the CD8/FOXP3 ratio, a finding associated with improved BCSS in TNBC.

Karihtala et al reported that 65% of women with TNBC have vimentin expressing invasive cancer cells (negative: 0-1%; 1+: 2-9%; 2+: 10-50%; 3+: over 50%) and that vimentin over-expression (2+/3+) is associated with poor breast cancer specific survival.[37] However, there are no data regarding the frequency of vimentin expression in TNBC with residual disease after standard NAC. Therefore, using a validated IHC antibody (Dako (M7020, clone 3B4) and as assessed in the Mayo CAP/CLIA laboratory, we identified a cohort of 30 Mayo clinic patients who underwent NAC with AC and taxane for TNBC (ER <1%, PR <1% and HER2 negative) and who had residual disease. Of these, 22 who FFPE sections available from residual disease at surgery, and 15/22 (68%) had strong vimentin staining (3+) in the residual tumor specimens. These data support the observation that EMT markers such as vimentin are highly expressed in TNBC resistant to chemotherapy.

In summary, diverse and newly described effects of CDK4/6i provide the rationale to prospectively study the pharmacodynamics of abemaciclib in TNBC using a “window” design in a chemotherapy resistant population of breast cancer patients treated either with or without pembrolizumab. Specifically, for TNBC patients who have completed NAC +/- pembrolizumab and have biopsy proven residual disease, we propose a window trial, to prospectively study the pharmacodynamics effects of abemaciclib on 1) CD8 T cells and FOXP3 regulatory cells (increase CD8/FOX3 ratio); 2) EMT pathway markers including vimentin, SNAIL/SLUG, TWIST, and pDUB3; 3) proliferation biomarkers (Ki-67); and 4) the expression of interferon-stimulated genes and genes involved in antigen presentation. Each of these parameters will be evaluated within the known TNBC molecular subtypes and based on known Rb status. The effects of abemaciclib will be evaluated in two different cohorts of patients treated either without pembrolizumab (Cohort A) or with pembrolizumab (Cohort B).

If our trial is successful, and abemaciclib is found to activate immune targeting T-Cells or inhibit metastases though direct effects on EMT in chemotherapy resistant TNBC,

there would be clear rationale for development of abemaciclib in the adjuvant treatment of TNBC.

3.5 Mayo Clinic Prospective Neoadjuvant Chemotherapy Study

Mayo investigators funded by the Center for Individualized Medicine and the Cancer Center developed a prospective clinical trial which incorporated treatment of high risk breast cancers with standard neoadjuvant chemotherapy along with collection of serial tumor samples for sequencing and development of PDX to further characterize tumor factors impacting response to chemotherapy.[3,38] The BEAUTY study enrolled 140 patients from March 2012 through May 2014 across all three (3) Mayo sites. Baseline tumor biopsies were obtained followed by treatment with a combination of taxane (with anti-HER2 therapy if HER2+ disease) and anthracycline/cyclophosphamide chemotherapy. Thirty-four percent of patients enrolled had TNBC. While 53% of the TNBC patients demonstrated pCR, 47% of patients had some degree of residual disease and therefore are at high risk of relapse.

3.6 Abemaciclib (Verzenio®)

There are currently 3 CDK 4/6 inhibitors that have demonstrated evidence for benefit in metastatic ER+, HER2- breast cancer. These include palbociclib, ribociclib, and abemaciclib, all of which are FDA approved for ER+ metastatic breast cancer.

3.6.1 Preclinical

Abemaciclib is a CDK4 and 6 inhibitor with three FDA indications for the treatment of hormone receptor-positive (HR+), human epidermal growth factor receptor 2 negative (HER2−) advanced or metastatic breast cancer (ABC): In combination with letrozole or anastrozole for postmenopausal women as initial endocrine−based therapy (MONARCH 3), In combination with fulvestrant for women with disease progression following endocrine therapy (MONARCH 2), and as a single agent for adult patients with disease progression following endocrine therapy and prior chemotherapy in the metastatic setting (MONARCH 1). This compound differs from both ribociclib and palbociclib in terms of its chemical structure and pharmacological characteristics.[39] It can be administered continuously and is able to cross the blood-brain barrier.

3.6.2 Clinical

Initial data regarding the clinical pharmacology, safety, and efficacy of abemaciclib in humans came from the JPBA study, an open-label, phase 1, dose escalation study, followed by tumor-specific expansion cohorts.[40] During the dose escalation phase, abemaciclib was administered at doses of 50–225 mg daily and 75– 275 mg twice daily. The recommended dose for phase II evaluation (RP2D) was 200 mg twice daily and a dose-proportional pharmacokinetic profile was noted, with a half-life ranging from 17 to 38 hours. Pharmacodynamic assessments were conducted in tumors and skin keratinocytes. Despite the long half-life, partial reversibility of abemaciclib-induced Rb phosphorylation and topoisomerase II-α expression in skin biopsies taken from patients treated once daily was observed. As the twice-daily

schedule demonstrated evidence of sustained target engagement over the dosing interval, this was further evaluated in the tumor-specific cohorts.

Fatigue was the dose-limiting toxicity of abemaciclib, which differed from that of palbociclib and ribociclib (neutropenia). Frequently observed treatment-related adverse events in the single-agent tumor-specific arms (n = 173) included fatigue (all grades = 41%; Grade 3 = 3%), diarrhea (all grades = 63%; Grade 3 = 5%), nausea (all grades = 45%; Grade 3 = 2%), vomiting (all grades = 25%; Grade 3 = 1%), and anorexia (all grades = 17%; Grade 3 = 0 %). Although diarrhea was a common side effect, it was effectively managed with prophylactic medications or a dose reduction. Hematologic side effects were less commonly observed when compared with palbociclib and ribociclib, e.g. leukopenia (all grades = 25%; Grade 3 = 10%), neutropenia (all grades = 23%; Grade 3 = 9%; Grade 4 = 1%), thrombocytopenia (all grades = 23%; Grade 3 = 7%), and anemia (all grades = 20%; Grade 3 = 4%). JPBA also included a monotherapy expansion cohort (n=47) of heavily pretreated metastatic breast cancer (MBC) patients (median of 7 prior lines of systemic therapy). Given the low-grade diarrhea that complicated the 200 mg twice-daily dose level, some patients received a lower starting dose of 150 mg twice daily which was associated with fewer treatment delays and dose reductions. Robust antitumor activity was observed including patients with HR-positive/HER2 negative breast cancer as well as HR+/HER2-positive MBC.

Preclinical data have demonstrated that abemaciclib effectively penetrates the BBB and improves survival outcomes in intracranial human brain tumor xenografts.[41] Therefore, paired cerebrospinal fluid (CSF) and unbound plasma concentrations of abemaciclib were procured from ten patients with glioblastoma participating in the JPBA trial. It was demonstrated that the CSF concentration of abemaciclib was similar to the plasma concentration of abemaciclib. Further, two patients with glioblastoma treated with abemaciclib achieved a prolonged PFS, which suggested that abemaciclib may be effective in the treatment of primary or metastatic brain tumors, and clinical trials are in progress. A phase II trial is studying the activity of abemaciclib in patients with brain metastases, including in a cohort of patients with HR+/HER2- advanced breast cancer.[42] Of 23 evaluable patients with HR+/HER2-MBC, two patients had confirmed durable partial responses in their brain lesions, and more patients are now being enrolled in the second phase of the study to further determine the potential benefit of CDK4/6i in patients with brain metastases. Of note, no responses were observed in patients with brain metastases secondary to HR+, HER2+ breast cancer.

The safety and efficacy of abemaciclib monotherapy was further evaluated in the phase 2 single-arm MONARCH 1 study (NCT02102490).[5] Women (n=132) with locally advanced or metastatic HR-positive, HER2-negative breast cancer whose disease had progressed following both endocrine therapy and 1-2 lines of chemotherapy in the metastatic setting were enrolled and treated with abemaciclib 200 mg every 12 hours until disease progression. The ORR was 19.7%, the clinical benefit rate (CR + PR + SD 6 months) was 42.4%, and median PFS was 6.0 months. The most frequently observed treatment-related adverse events were: creatinine elevation (all grades = 98.5%; grade 3 = 0.8%), diarrhea (all grades =

90.2%; Grade 3 = 19.7%), neutropenia (all grades = 87.7%; Grade 3/4 26.9%) and fatigue (all grades = 65.2%; Grade 3 = 12.9%). As abemaciclib is a competitive inhibitor of creatinine efflux transporters (OCT2, MATE1, and MATE2-K), cystatin C calculated glomerular fraction rate was performed and values were within normal limits. Notably, abemaciclib-induced diarrhea was manageable with antidiarrheal medication or dose reduction, and discontinuation of therapy due to side-effects was infrequent (7.6%). Notably, the results of this trial resulted in FDA approval.

Several trials have evaluated the combination of abemaciclib and endocrine therapy, including the JPBA trial (abemaciclib 200 mg twice daily combined with fulvestrant[40] and the phase 1b JPBH study (NCT02057133). In the latter, the safety, pharmacokinetics, and antitumor activity of abemaciclib in combination with various endocrine therapies and HER2-directed therapy has been evaluated. Participants were allocated to one of six cohorts and received either abemaciclib plus letrozole 2.5 mg daily (part A), anastrozole 25 mg daily (part B), tamoxifen 20 mg daily (part C), exemestane 25 mg daily (part D), exemestane 25 mg daily plus everolimus 5 mg daily (part E), or trastuzumab 6–8 mg/kg IV once every 21 days (part F). Participants in parts A–E had HR-positive, HER2-negative MBC and those in part F had HER2-positive MBC. Patients in parts A-E could not have received prior chemotherapy in the metastatic setting, however one prior line of chemotherapy was allowed for metastatic disease in part F. Preliminary data from the first 65 patients included in parts A–D have been presented and demonstrated that the most common treatment- related adverse events were diarrhea (all grades = 95%; Grade 3 = 31%), fatigue (all grades = 71%; Grade 3 = 14%), nausea (all grades = 62%; Grade 3 = 6%), and neutropenia (all grades = 31%; Grade 3 = 17%). Diarrhea was manageable with antidiarrheal agents or dose reduction. The disease control rate (CR + PR + SD) was 67% for parts A + B (non-steroidal aromatase inhibitors (36 patients), including 2 confirmed PRs, and 75% for part C (tamoxifen 16 patients). Updated results from this study were recently reported. In part F, eleven patients had HR-positive HER2-positive MBC and seven patients had HR-negative, HER2-positive MBC. Interestingly, although no complete responses (CR) or partial responses (PR) were noted in the patients with HR-negative, HER2-positive disease, four patients achieved stable disease (SD). Further, in the 7 patients who were treated with trastuzumab and a 150mg dose of abemaciclib, the disease control rate (CR + PR + SD) was comparable in patients with HR-positive and HR-negative disease (54.5 % vs. 57.1 %, respectively). These data suggest that further study of abemaciclib in ER-negative breast cancer is warranted.

In the FDA registration phase III double-blind MONARCH 2 study , 669 patients with HR-positive, HER2-negative advanced breast cancer who had progressed while receiving neoadjuvant/adjuvant endocrine therapy ET, ≤12 months from the end of adjuvant ET, or while receiving first-line ET for metastatic disease, were enrolled.[10] Patients were randomized in a 2:1 fashion to receive fulvestrant (standard dosing) along with either abemaciclib (150 mg twice daily) or placebo. The combination of abemaciclib and fulvestrant significantly extended PFS versus fulvestrant alone (median, 16.4 v 9.3 months; hazard ratio, 0.553; 95% CI, 0.449 to

0.681; P <0.001) with higher response rates observed in patients with measurable disease (48.1% (95% CI, 42.6% to 53.6%) vs. 21.3% (95% CI, 15.1% to 27.6%). The most frequent adverse events in the abemaciclib versus placebo arms were diarrhea (86.4% vs 24.7%), neutropenia (46.0% vs 4.0%), nausea (45.1% vs 22.9%), and fatigue (39.9% vs 26.9%). In 2019, a formal survival analysis was performed. Patients randomized to abemaciclib plus fulvestrant had superior overall survival compared to fulvestrant alone (HR 0.757; p=0.0137; 46.7 months vs 37.3 months). Most notably, in patients with primary endocrine resistance, abemaciclib extended survival compared to the placebo arm by 7.2 months (HR 0.686; 38.7 months vs 31.5 months).[56]

The double-blind phase III MONARCH 3 FDA Registration trial randomized 493 patients to 150 mg of abemaciclib or placebo twice daily combined with either 1 mg of anastrozole or 2.5 mg of letrozole daily until disease progression or unacceptable toxicity.[9] The study included postmenopausal women with locoregionally recurrent or metastatic breast cancer and no prior systemic treatment for advanced disease. Median PFS was increased in those who received abemaciclib (P = .000021; median: not reached in the abemaciclib arm, 14.7 months in the placebo arm). For participants with measurable disease, the objective response rate (ORR) was 59% in the abemaciclib group and 44% in the placebo arm (P = .004). Diarrhea was the most frequent adverse effect (81.3%) observed in patients who received abemaciclib, but was usually Grade 1 (44.6%). With regards to differences in toxicity between patients who received abemaciclib vs. placebo, the most frequent Grade 3 or 4 adverse events were neutropenia (21.1% v 1.2%), diarrhea (9.5% v 1.2%), and leukopenia (7.6% v 0.6%). The most common AEs overall (of any grade) were diarrhea, neutropenia, fatigue, and nausea. Notably, there was an increased incidence of venous thromboembolic disease in the abemaciclib arm (in 16 (4.9%) of patients versus one (0.6%) in the placebo arm).[9,10,43] Safety of CDK4/6 inhibitors in the pre-operative window setting.

A recent palbociclib window study demonstrated that 2 weeks of treatment was safe with no delay in surgery, and palbociclib significantly decreased Ki-67.[44]

3.7 Assessing response to neoadjuvant chemotherapy

Neoadjuvant chemotherapy decreases tumor burden in the breast and the axillary lymph nodes. Assessment of the extent of residual disease after neoadjuvant chemotherapy is critical to evaluate response to chemotherapy and to guide surgical decision making. Pathologic complete response (pCR) defined as no residual invasive disease in the breast or the lymph nodes occurs in approximately 30-40% of all cases[45]with higher rates of approximately 40-50% in patients with TNBC.[46]

Assessment of extent of residual disease in the breast can be performed using mammogram, ultrasound and/or MRI. Ultrasonography is more accurate than clinical breast examination or mammography for assessing residual tumor size following NAC.[47] When both mammogram and ultrasound demonstrate no residual disease the likelihood of a pCR was 80 %.[48] Breast MRI has the greatest accuracy in assessing extent of residual disease. However MRI also may overestimate and underestimate the presence and extent of residual disease. At the current time,

given the limitations of breast imaging to reliably demonstrate the presence or absence of residual disease after NAC, histological evaluation of the tumor bed is standard of care. In this study, a percutaneous biopsy will be performed to obtain tumor tissue to prove there is residual invasive disease and identify patients that would candidates for the neoadjuvant window study. In order to increase the likelihood of identifying the area of the tumor biopsied at pre-registration, a clip will be placed at the time of the biopsy. Identification of the clip and collection of tumor tissue around the clip will be mandated to increase the likelihood of collection of tumor in the same region (pre/post abemaciclib).

3.8 Trial Summary

Nearly all patients with TNBC are being treated with neoadjuvant chemotherapy either with or without pembrolizumab [ref: PMID: 32101663]. However, multiple studies have demonstrated that TNBC patients with residual disease after receipt of standard neoadjuvant based chemotherapy have high rates of distant recurrence and death within 3 years. Therefore, prioritization of drugs that target this group of patients is of high priority.

The neoadjuvant window trial design has been successfully utilized to develop multiple drugs such as aromatase inhibitors. However, the use of a window design to test drugs in patients with chemotherapy resistant disease has not been explored. A major issue for developing drugs in the post-neoadjuvant space relates to the lack of tissue for examining drug response phenotypes. The development of a "window" trial in the post-neoadjuvant space allows the interrogation of tumor tissue that is resistant to chemotherapy and provides the opportunity to study biomarkers associated with critical drug response phenotypes. Therefore, the use of a post-neoadjuvant window is novel, and provides an important opportunity to develop a new approach to drug development.

Abemaciclib is FDA approved for the treatment of estrogen receptor positive, HER2 negative metastatic breast cancer. However, multiple publications suggest that drugs within this class may have antitumor effects through a broad range of mechanisms, including effects on immune activation, effects on EMT, and proliferation (e.g. for LAR breast cancer with intact Rb). Therefore, our proposed study designed to assess abemaciclib response phenotypes in TNBC resistant to chemotherapy and/or immune checkpoint therapy, could provide critical information for development of larger studies to evaluate the antitumor activity of abemaciclib in TNBC. For example, if abemaciclib is found to activate immune signatures, alter tumor signatures associated with distant metastases, or inhibit proliferation, abemaciclib may demonstrate to be an important adjuvant treatment to improve the survival of women with chemotherapy resistant TNBC.

3.9 Lowering of Starting Dose of Abemaciclib

The dosing and adverse event data of the first 4 patients treated with abemaciclib were reviewed in early May 2021. Two of these 4 patients discontinued treatment due to adverse events after 12 and 14 days of treatment. The first patient discontinued treatment (5 days prior to surgery) having developed Grade 2 diarrhea

and vomiting with Grade 1 nausea, fatigue, and anorexia. The second patient discontinued treatment (8 days prior to surgery) having developed Grade 1 diarrhea, nausea, alanine aminotransferase increase, and platelet count decrease. Given that the goal of this window study is to investigate the pharmacodynamic effects of abemaciclib in surgically resected tumor tissue, adherence to abemaciclib dosing up to the day prior to surgery is critical to meet the trial endpoints. Thus, given these tolerability issues, the protocol was amended to lower the starting dose of abemaciclib from 200mg every 12 hours to 150 mg every 12 hours.

3.10 Addition of Cohort B (evaluating effects of abemaciclib in TNBC patients treated with pembrolizumab and NAC)

Based on the preclinical data outlined above, phase I studies were conducted to evaluate the toxicities and antitumor activity of the combination of abemaciclib and pembrolizumab [DOI: 10.1158/1538-7445.AM2020-CT108]. However, further clinical development was halted because of high rates of Grade 3/4 toxicity when the drugs were combined. In spite of this issue, there remains strong rationale to study abemaciclib sequentially in TNBC patients who exhibit residual disease after receipt of combination NAC and pembrolizumab. Therefore, we will add a second cohort of TNBC patients who were treated with NAC plus pembrolizumab, based on the recent FDA approval of the use of pembrolizumab along with NAC as previously published in the Keynote 522 study [https://www.nejm.org/doi/full/10.1056/NEJMoa1910549].

4. PARTICIPANT SELECTION

4.1 Pre-Registration Inclusion Criteria

4.1.1 Women of age ≥18 years. (NOTE: Rationale provided in Section 17.8.)

4.1.2 Clinical T1-4, N0-3, M0 breast cancer at diagnosis (prior to the start of neoadjuvant chemotherapy) by AJCC staging version 8.

Note: Benign breast disease, lobular carcinoma in situ (LCIS) or ductal carcinoma in situ (DCIS) in the ipsilateral or contralateral breast is allowed.

Note: Additional ipsilateral or contralateral invasive breast cancer is allowed. The index lesion is the largest triple-negative, chemotherapy-resistant lesion.

4.1.3 Histological confirmation of triple negative invasive breast cancer (defined as ER≤10%, PR≤10% and HER2 not amplified by ISH or IHC 0/1) at diagnosis.

4.1.4 Neoadjuvant chemotherapy (NAC)

4.1.4.1 Cohort A: **CLOSED TO PRE-REGISTRATION and REGISTRATION as of MCCC Amendment 6**
Neoadjuvant chemotherapy (NAC) with one of the following regimens that was not discontinued early due to intolerability with less than 50% of planned treatment given due to disease progression or patient request:

- Paclitaxel or docetaxel followed by one of the following: the combination of doxorubicin and cyclophosphamide (AC); the combination of epirubicin and cyclophosphamide (EC) or the combination of 5-fluorouracil, epirubicin and cyclophosphamide (FEC)
  Note: Carboplatin may be added to these regimens
- AC or EC or FEC followed by docetaxel or paclitaxel
  Note: Carboplatin may be added to these regimens
- Docetaxel in combination with doxorubicin and cyclophosphamide (TAC)
- Docetaxel in combination with cyclophosphamide (TC) (for patients who are not candidates for anthracyclines)
- Carboplatin or cisplatin in combination with a taxane (paclitaxel, docetaxel, or nab-paclitaxel) (for patients who are not candidates for anthracyclines)

4.1.4.2 Cohort B: Neoadjuvant chemotherapy (NAC) with one of the following regimens in combination with pembrolizumab that was not discontinued early due to intolerability with less than 50% of planned

treatment given due to disease progression or patient request:

- Paclitaxel or docetaxel followed by one of the following: the combination of doxorubicin and cyclophosphamide (AC); the combination of epirubicin and cyclophosphamide (EC) or the combination of 5-fluorouracil, epirubicin and cyclophosphamide (FEC)
  Note: Carboplatin may be added to these regimens
- AC or EC or FEC followed by docetaxel or paclitaxel [Note: Carboplatin may be added to these regimens]
- Docetaxel in combination with doxorubicin and cyclophosphamide (TAC)
- Docetaxel in combination with cyclophosphamide (TC)
- Carboplatin or cisplatin in combination with a taxane (paclitaxel, docetaxel, or nab-paclitaxel)

4.1.5 Residual lesion/enhancement seen in the breast on breast imaging performed after completion of NAC.

4.1.6 Able to swallow oral medication.

4.1.7 Willing to undergo biopsy for research (Sections 5 and 10).

4.1.8 Willing to provide tissue and blood samples for correlative research purposes (see Section 10).

4.1.9 Willing to stop use of strong and moderate inducers and/or strong inhibitors of cytochrome P450 3A ≤7 days prior to registration.

4.1.10 Provide written informed consent.

4.2 Pre-Registration Exclusion Criteria

4.2.1 History of deep venous thrombosis (DVT) or pulmonary embolisms (PE) ≤12 months prior to preregistration; OR
Active DVT and/or PE requiring anti-coagulant therapy.
NOTE: Patients who are on anti-coagulant therapy for maintenance are eligible as long as the DVT and/or PE was >12 months prior to enrollment and there is no evidence for active thrombosis (either DVT or PE).
NOTE: Patients on anticoagulation are eligible; however peri-biopsy and

peri-surgical management of anticoagulation is per the institutional standard of care.

4.2.2 Prior treatment with CDK 4/6 inhibitors (e.g. palbociclib, ribociclib, abemaciclib, etc.)

4.2.3 Prior treatment with radiation for this breast cancer.

4.2.4 Prior incisional or excisional breast biopsy for this cancer.

4.2.5 Any contraindications to pre-registration biopsy (such as bleeding diatheses, etc.).

4.2.6 Receiving any investigational agent which would be considered as a treatment for the primary neoplasm.

4.2.7 Other active malignancy ≤3 years prior to registration.
EXCEPTIONS: Non-melanotic skin cancer or carcinoma-in-situ of the cervix.
NOTE: If there is a history of prior malignancy, they must not be receiving another specific treatment for prior malignancy.

4.2.8 Biopsy proven Stage IV breast cancer.

4.2.9 Serious pre-existing medical conditions that 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 Crohn's disease or ulcerative colitis or a preexisting chronic condition resulting in baseline Grade 2 or higher diarrhea).

4.2.10 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)
- Sudden cardiac arrest

NOTE: Patients on anticoagulation are eligible; however peri-biopsy and peri-surgical management of anticoagulation is per the institutional standard of care.

4.3 Registration - Inclusion Criteria for All Patients

4.3.1 Registration must occur ≤56 days after last dose of NAC.

4.3.2 ECOG Performance Status (PS) 0, 1, or 2 (Appendix A)

4.3.3 Cohort B Group 4 ONLY:

4.3.3.1 The following laboratory values obtained after completion of NAC but ≤14 days prior to registration:

- Absolute neutrophil count (ANC) ≥1500/mm^3

- Platelets (PLT) ≥100,000/mm^3
- Hemoglobin (HgB) ≥8.0g/dL
- Total bilirubin ≤1.5 x ULN
- Aspartate transaminase (AST)(SGOT) ≤3 x ULN
- Alanine aminotransferase (ALT)(SGPT) ≤3 x ULN
- Serum creatinine ≤1.5 x ULN

4.3.3.2 Negative pregnancy test done ≤7 days prior to registration, for persons of childbearing potential only.

4.4 Registration - Exclusion Criteria for Cohort B Group 4 only

4.4.1 Any of the following because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown:

- Pregnant persons
- Nursing persons
- Persons of childbearing potential who are unwilling to employ adequate contraception

4.4.2 Failure to recover to Grade 1 or lower from effects of neoadjuvant chemotherapy.
Exceptions: Residual alopecia and Grade 2 peripheral neuropathy are allowed.

4.4.3 Concurrent use of strong and moderate inducers and/or strong inhibitors of cytochrome P450 3A ≤7 days prior to registration.

4.4.4 Known infections as follows (NOTE: Screening is not required for enrollment):

- Active systemic bacterial infection requiring intravenous antibiotics
- Active fungal infection (requiring intravenous or oral antifungal treatment)
- Detectable viral infections (e.g., known HIV, known active hepatitis B or C)

4.4.5 Concurrent use of chemotherapy, radiotherapy, immunotherapy, or other components of neoadjuvant treatment.
NOTE: Patients must complete all elements of NAC ≥21 days prior to starting abemaciclib.

4.5 Inclusion of Underrepresented Populations

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age or race in the clinical trial outlined. This trial is open to the accrual of women only.

5. REGISTRATION PROCEDURES

5.1 Grouping Factors for Eligiblity Checklist

5.1.1 Disease group: No invasive disease (Group 3) vs. Invasive disease (Group 4)
NOTE: Groups 1 and 2 are closed as of Amendment 6

5.1.2 Cohort: Cohort A (neo-adjuvant treatment did not include pembrolizumab) vs. Cohort B (neo-adjuvant treatment included pembrolizumab)
NOTE: Cohort A is closed as of Amendment 6

5.2 Pre-Registration (Step 0)

To pre-register a patient, access the Mayo Clinic Research Registration Application at https:\\registration.mayo.edu. The Research Registration Application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the Research Registration Office at (507) 284-2753 or random01@mayo.edu between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for the Research Registration Application are available on the Office of Clinical Trials web page ((https://www.mayo.edu/research/centers-programs/center-clinical-translational-science/offices/office-of-clinical-trials/research-registration-application) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the Research Registration Application can be confirmed in any of the following ways:

- Contact the Research Registration Office (507) 284-2753 or random01@mayo.edu. If the patient was fully registered, the Research Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

5.2.1 Verification at pre-registration

Prior to accepting the pre-registration, the registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient pre-registration eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information.

5.2.2 Pre-registration tests/procedures

Pre-registration tests and procedures (see Section 6.0) must be completed within the guidelines specified on the test schedule.

5.3 Registration Procedures

5.3.1 Registration

To register a patient, access the Mayo Clinic Research Registration Application at https:\\registration.mayo.edu. The Research Registration Application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the Research Registration Office at (507) 284-2753 or random01@mayo.edu between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the Research Registration Application are available on the Office of Clinical Trials web page ((https://www.mayo.edu/research/centers-programs/center-clinical-translational-science/offices/office-of-clinical-trials/research-registration-application) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the Research Registration Application can be confirmed in any of the following ways:

- Contact the Research Registration Office (507) 284-2753 or random01@mayo.edu. If the patient was fully registered, the Research Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

5.3.2 Correlative Research

A mandatory tissue and blood correlative research component is part of this study. The patient will be automatically registered onto this component (see Section 10).

5.3.3 Documentation

Documentation of IRB approval must be on file in the Research Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Research Registration Office (fax: 507-284-0885 or email: random01@mayo.edu). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Research Registration Office is no longer necessary.

5.3.4 Verification at registration

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

5.3.5 Patient permissions

At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research on cancer.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn about, prevent, or treat other health problems.
- Patient has/has not given permission for someone to contact them about future participation in more research

5.3.6 Additional Qualifications

5.3.6.1 Treatment on this protocol must commence at Mayo Clinic under the supervision of a medical oncologist.

5.3.6.2 Treatment cannot begin prior to registration and must begin ≤14 days after registration.

5.3.6.3 Pretreatment tests/procedures (see Section 6) must be completed within the guidelines specified on the test schedule. Prior to registration a research biopsy of breast tissue must be taken for central pathologic review.

5.3.6.4 All required baseline symptoms must be documented and graded.

5.3.6.5 **Cohort B Group 4** ONLY: Study drug is available on site.

5.3.6.6 Blood draw kit is available on site.

5.3.6.7 Stool kit is available on site.

6. STUDY CALENDAR

6.1 Cohort B GROUP 3 Study Calendar – No invasive disease

Parameter	Pre-Reg	Registration [1] (Baseline)	At time of surgery
Demographics		X	
CLINICAL EVALUATIONS:			
History and Physical including vital signs[2], weight, PS		X	
Height		X	
LABORATORY/RADIOLOGIC EVALUATIONS:			
Hematology (CBC with diff)	X[3]		
Tumor measurement (breast imaging) and report[4]	X		
CORRELATIVE STUDIES:			
Research Tumor/Tissue Biopsy	X		
Research tissue specimens from clinical procedures	X[5]		X
Research blood specimens		X[6]	
Research stool specimens		X[7]	
ADDITIONAL INFORMATION:			
Submission of Surgical (Operative) and Pathology Reports	X[8]		X

[1] ≤14 days prior to registration
[2] Vital signs include blood pressure, pulse, temperature
[3] After completion of NAC and ≤3 days prior to biopsy to rule out Grade 4 neutropenia
[4] Tumor measurement/breast imaging should be per institutional standard of care. Bi-dimensional measurement should be collected. Imaging reports from diagnosis and post-NAC should be submitted for all patients during pre-registration.
[5] Collection of a representative block or slides from initial diagnostic biopsy (prior to neoadjuvant chemotherapy)
[6] Research blood draw must be scheduled (paid by study); May be drawn after patient consents to the study and pre-registration patient study ID is available
[7] Baseline stool collection kit may be given to patient any time after consent is obtained and pre-registration patient study ID is available.
[8] Submit reports from biopsy at the time of diagnosis as well as reports from Pre-Registration research biopsy for this study

6.2 Cohort B GROUP 4 Study Calendar – Residual invasive disease

Parameter	Pre-Reg	Registration[9] (Baseline)	Abemaciclib 14-21 days prior to surgery	Prior to surgery	At time of surgery	End of Study 30-60 days after surgery[10]
Demographics		X				
CLINICAL EVALUATIONS:						
History and Physical including vital signs[11], weight, PS		X		X		
Height		X				
LABORATORY/RADIOLOGIC EVALUATIONS:						
Hematology (CBC with diff)	X[12]	X		X[13]		
Chemistries[14]		X		X		
Pregnancy Test (serum or urine)[15]		X				
Tumor measurement (breast imaging) and report[16]	X					
TREATMENT ADMINISTRATION:						
Abemaciclib			X			

[9] ≤14 days prior to registration
[10] Telephone contact approximately 30-60 days after definitive surgery (breast tumor resection)
[11] Vital signs include blood pressure, pulse, temperature
[12] After completion of NAC and ≤3 days prior to biopsy to rule out Grade 4 neutropenia
[13] CBC with differential ≤3 days prior to surgery (This panel and venipuncture are covered by research.)
[14] Serum chemistry includes a complete metabolic panel (CMP) [CPT 80053]
[15] For persons of childbearing potential only, Must be done ≤7 days prior to registration, and, if start of treatment is >7 days after initial testing, again ≤7 days prior to start of treatment.
[16] Tumor measurement/breast imaging should be per institutional standard of care. Bi-dimensional measurement should be collected. Imaging reports from diagnosis and post-NAC should be submitted for all patients during pre-registration.

Parameter	**Pre-Reg**	**Registration[9] (Baseline)**	**Abemaciclib** 14-21 days prior to surgery	**Prior to surgery**	**At time of surgery**	**End of Study** 30-60 days after surgery[10]
CORRELATIVE STUDIES:						
Research Tumor/Tissue Biopsy	X				X[17]	
Research tissue specimens from clinical procedures	X[18]				X	
Research blood specimens		X		X[19]		
Research stool specimens		X[20]		X[21]		
ADDITIONAL INFORMATION:						
Adverse Events/Symptom Assessment		X		X		X
Patient Medication Diary[22]			X			
Submission of Surgical (Operative) and Pathology Reports	X[23]				X	

Note: Additional tests may be performed at the discretion of the treating investigator as clinically indicated.

[17] If the patient will not have surgery, a research biopsy should be performed 14-21 days after starting study drug.
[18] Collection of a representative block or slides from initial biopsy (prior to neoadjuvant chemotherapy)
[19] Collect research blood after completion of abemaciclib, prior to surgery
[20] Baseline stool collection kit may be given to patient any time after consent is obtained and pre-registration patient study ID is available. Preferred timeline is ≤14 days prior to C1D1
[21] Pre-surgery stool kit can be collected up to 5 days prior to surgery
[22] NOTE: Study staff upload completed diary to Medidata Rave
[23] Submit reports from biopsy at the time of diagnosis as well as reports from Pre-Registration research biopsy for this study

7. TREATMENT PLAN

Surgery should be planned/scheduled for no later than 12 weeks after the last dose of neoadjuvant chemotherapy.

7.1 Biopsy during preregistration phase

Breast biopsy to obtain tissue from residual tumor/tumor bed. Biopsies should be obtained from any residual abnormal tissue or if no tumor visible, biopsy around clip. If the research biopsy is adjacent to the existing clip and the clip is NOT removed, then no additional clip needs to be placed.

If the research biopsy removes the existing clip, OR if the area biopsied is >1cm from the clip, then a clip should be placed to mark the area of the research biopsy. [In the situation where a second clip is placed, recommendation is to use a different shaped clip than the one already present in the breast].

NOTE: Ultrasound-guided biopsy obtain 6-8 cores using 14 gauge needle.
If no target seen on ultrasound, then stereotactic biopsy should be performed obtaining 4-6 cores using 9 gauge needle.

First and second core will be FFPE. The first core will be processed locally for H&E and used for eligibility. The H&E as well as the remaining portion of Core 1 and all of Core 2 will be sent to Mayo Clinic in Rochester, MN, for research purposes.

The remaining cores will be snap frozen (with the exception of Cores 5 and 6 at Mayo Rochester only which will be processed for PDX and organoids). Please see lab manual for specimen processing details.

7.2 Cohort B Group 4: Abemaciclib Administration

Agent administered orally twice daily for one cycle.

Agent	Dose Level	Route	Day	ReRx
abemaciclib	150mg	Oral (po) twice per day	at least Days 1-14 at most Days 1-21*	NA

*Length of treatment depends on when surgery is scheduled. Last dose should be taken one day prior to surgery.

Abemaciclib cannot be started until ≥21 days after last dose of neoadjuvant chemotherapy, and ≥21 days after the last dose of pembrolizumab.

If drug is stopped or delayed for any reason other than life threatening adverse event(s), patients may resume abemaciclib per protocol and may receive up to 42 total doses of drug prior to surgery.

7.3 Cohort B Group 4 Concomitant Treatment and Supportive Care Guidelines

7.3.1 Supportive care

Patients should receive standard supportive care during treatment with abemaciclib. This treatment includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions.
All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

7.3.2 Diarrhea

NOTE: For this study, patients will be given a prescription for loperamide at the same time as the abemaciclib is dispensed.

In the event of diarrhea, supportive measures should be initiated as early as possible. These include the following:

- At the first sign of loose stools, the patient should initiate anti-diarrheal therapy (e.g. loperamide) and notify the investigator for further instructions and appropriate followup.
- Patients should also be advised to drink fluids (e.g., 8 to 10 glasses of clear liquids per day).
- If diarrhea does not resolve with anti-diarrheal therapy within 24 hours to either baseline or Grade 1, then patient should contact study staff and dosing should be adjusted as outlined in Section 8.4.2. In the event of a weekend, patient will be instructed to contact physician on call.
- Site personnel will assess response to adjusted anti-diarrheal therapy within 24 hours.

7.3.3 Hepatic Monitoring

Details for hepatic monitoring depend upon the severity and persistence of observed laboratory test abnormalities. To ensure patient safety the investigator should collect specific recommended clinical information and follow-up laboratory tests as shown in Appendix C: Lilly Guidance for Hepatic Treatment Emergent Abnormality.

7.4 Duration of Therapy

7.4.1 Cohort B Group 3: Patients without residual disease

Patients whose pre-registration biopsy specimen has no residual invasive disease identified on the H&E will proceed to surgery as scheduled.

7.4.2 Cohort B Group 4: Patients with residual invasive disease

Patients who have residual invasive disease identified on H&E will receive abemaciclib for a minimum of 14 days and a maximum of 21 days such that the last dose of abemaciclib is administered the day prior to surgery.

It is strongly recommended that the surgery date is scheduled and the date of initiation of abemaciclib calculated to be 15-22 days prior to date of surgery,

so that the patient receives a minimum of 14 days and a maximum of 21 days of treatment, with the goal that the last dose is administered the day prior to surgery.

The reasons for discontinuation of abemaciclib include:

- Treatment intolerability defined as:
  ANC <1,000/mm^3, platelets <100,000/mm^3, or any ≥Grade 2 treatment-related adverse events
- Intercurrent illness
- Administration of alternative anti-cancer treatment
- Request by patient to discontinue study treatment
- Withdrawal of consent
- Found to be ineligible after starting abemaciclib

All reasons for discontinuation of therapy should be documented clearly in the medical record.

If a subject discontinues treatment early and goes to surgery without receiving other anti-cancer therapy, surgical tissue/tumor and research blood will still be collected.

7.5 Duration of Follow-Up

1) Cohort B Group 3: Patients who undergo a pre-registration biopsy with no residual invasive disease identified on the H&E will proceed to surgery as scheduled. Surgical, imaging, and pathology reports will be submitted and patients will go off study after submission of reports.

2) Cohort B Group 4: Patients who undergo a pre-registration biopsy and have residual invasive disease identified on the H&E, and who receive any amount of study drug will be followed for adverse events until approximately 30-60 days post-surgery. Submission of tumor tissue, and pathology and imaging reports should occur as outlined in Section 6.

7.6 Criteria for Study Discontinuation

- Study participation is complete upon withdrawal of consent.
- Patients whose pre-registration biopsy has residual invasive disease identified on the H&E but refuse to start treatment with abemaciclib are considered a cancel. All onstudy materials and end of treatment case report form are to be completed.

7.7 Treatment Following Surgery

Following surgery, patients should proceed with standard guideline recommended adjuvant treatments.

8. EXPECTED ADVERSE EVENTS AND DOSING DELAYS/DOSE MODIFICATIONS –COHORT B GROUP 4

8.1 Treatment Administration

Abemaciclib will be administered orally at a dose of 150 mg (3 x 50mg tablets) twice daily for 14-21 days.

Abemaciclib should be taken with food. (NOTE: Grapefruit and grapefruit juice should be avoided while taking abemaciclib.)

If a patient misses a dose, she must be instructed not to make it up but just take her next regular dose.

If a patient vomits any time after taking a dose, she must be instructed not to make it up but to resume the next regular dose.

If a patient inadvertently takes an extra dose during a day, instruct patient to call her physician for instructions.

CBC with diff is to be performed 3 days (±1 day) prior to surgery. Treatment with abemaciclib should be discontinued if ANC <1,000/mm^3, platelets <100,000/mcL, or patients present with any ≥Grade 2 treatment-related adverse events.

If a patient needs to stop treatment prematurely, she will have surgery on the scheduled date unless contraindicated (see Section 8.3.2). Surgery may be rescheduled at the discretion of the treating physician.

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. Therefore, drugs that are strong and moderate inducers of CYP3A and/or strong inhibitors of CYP3A should be avoided or substituted if necessary.

8.2 Anticipated Adverse Events

The primary short term adverse events related to abemaciclib include neutropenia (Grade 1-3), diarrhea, nausea, and fatigue (Grade 1-2). Given the short term nature of this study, ≥Grade 3 adverse events are not anticipated. However, in the event these adverse events are observed, recommendations for management are included below.

8.2.1 Assessment of renal insufficiency

Elevation of serum creatinine is observed with abemaciclib, and is due to a pharmacological inhibitory effect of abemaciclib on renal tubular transporters without affecting glomerular function. The rise in serum creatinine (mean increase, 0.2 mg/dL) occurs within the first 28-days of abemaciclib, and and is reversible upon treatment discontinuation. Alternative markers (such as BUN, cystatin C level, or cystatin C calculated GFR), which are not based on creatinine, may be considered to determine whether renal function is impaired.

8.3 Management of Adverse Events

8.3.1 Diarrhea

At enrollment, patients should receive instructions on the prompt management of diarrhea. Patients should be given a prescription for loperamide at the same time as the abemaciclib is dispensed. In the event of diarrhea, supportive measures should be initiated as early as possible.

At the first sign of loose stools, the patient should initiate anti-diarrheal therapy, and notify the investigator for further instructions and appropriate follow up. If diarrhea does not resolve with anti-diarrheal therapy within 24 hours to either baseline or Grade 1, then patient should contact study staff and dose adjustments should be made as outlined in Section 8.0. Patients should be advised to drink fluids (e.g. 8 to 10 glasses of clear liquids per day).

Diarrhea could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of Grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (Grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting should be hospitalized for intravenous hydration and correction of electrolyte imbalances.

8.3.2 Other Adverse Events

Any Grade 3 or 4 adverse event, regardless of attribution, should result in drug discontinuation. Surgery should be postponed until all adverse events are resolved to <Grade 3.

Note: Surgery in the setting of ≤Grade 2 neutropenia is allowed, given that neutrophil recovery is rapid after stopping abemaciclib (see Investigator Brochure).

8.4 Dose Modifications/ Delays

8.4.1 Dose Adjustments for Abemaciclib

Dose Level	Abemaciclib
0*	150 mg every 12 hours
-1	100 mg every 12 hours
-2	50 mg every 12 hours

*Starting dose level

Study drug must be discontinued if further dose reduction is required beyond 50mg every 12 hours.

Study drug should be discontinued if adverse events of ≥Grade 2 do not resolve within 7 days of onset.

If study drug is omitted for >7 days, then permanently discontinue study drug.

NOTE: Specific dose modifications/delays are in the subsections below.

8.4.2 Dose Modifications for Diarrhea

CTCAE Grade	Abemaciclib Dose Modifications
Grade 1	No dose modification is required.
Grade 2	If AE does not resolve within 24 hours to ≤Grade 1, omit dose for up to 7days. Dose reduction is not required.
Grade 2 that persists or recurs after resuming the same dose despite maximal supportive measures	Omit dose until AE resolves to ≤Grade 1 for up to 7 days Resume at next lower dose
Grade 3 or 4 or requires hospitalization	

8.4.3 Dose Modifications for Increased Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

CTCAE Grade	Abemaciclib Dose Modifications
Grade 1 (>ULN-3.0 x ULN) Grade 2 (>3.0-5.0 x ULN)	No dose modification is required.
Persistent or Recurrent Grade 2, or Grade 3 (>5.0-20.0 x ULN) that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Omit dose until AE resolves to baseline or Grade 1 for up to 7 days. Resume at next lower dose.
≥Grade 2 (>3.0 x ULN) with **total bilirubin** >2 x ULN, in the absence of cholestasis	Discontinue abemaciclib
Grade 4 (>20.0 x ULN)	Discontinue abemaciclib.

To ensure patient safety the investigator should collect specific recommended clinical information and follow-up laboratory tests as shown in Appendix C.

8.4.4 Dose Modifications for Infection, Bleeding, Anemia, Neutropenia and Other Cytopenias

CTCAE Grade	Abemaciclib Dose Modifications
Grade 1 or 2	No dose modification is required.
Grade 3	Omit dose until AE resolves to ≤Grade 2. Dose reduction is not required.
Grade 3, recurrent, or Grade 4	Omit dose until AE resolves to ≤Grade 2. Resume at next lower dose.
Patient requires administration of a blood cell growth factor	Omit abemaciclib dose for at least 48 hours after the last dose of blood cell growth factor and until AE resolves to ≤Grade 2. Resume abemaciclib at next lower dose unless the dose was already reduced for the AE that led to the use of the growth factor.

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

Abemaciclib has been shown to increase serum creatinine due to inhibition of renal tubular transporters without affecting glomerular function (as measured by iohexol clearance). In clinical studies, increases in serum creatinine occurred within the first month of abemaciclib dosing, remained elevated but stable through the treatment period, were reversible upon treatment discontinuation, and were not accompanied by changes in markers of renal function, such as blood urea nitrogen (BUN), cystatin C, or calculated glomerular filtration rate based on cystatin C.

8.4.6 Dose Modifications for Interstitial Lung Disease (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	Omit dose until toxicity resolves to baseline or Grade 1. Resume at next lower dose.
Grade 3 or 4	Discontinue abemaciclib.

8.4.7 General Guidance for Venous Thromboembolic Events

VTE has been identified as an adverse drug reaction (ADR) for abemaciclib in combination with endocrine therapy. However, in studies with single-agent abemaciclib use in the metastatic breast cancer or other tumor types, including NSCLC, no increased rates of VTEs were observed as compared to the incidence of VTEs for these particular patient populations who were treated with other anticancer agents. At this time, the mechanism underlying the association between abemaciclib and the occurrence of VTEs is not known. Venous thromboembolic events have been reported with other CDK4 and 6 inhibitors, and ET is known to be associated with the occurrence of VTEs. Monitor patients for signs and symptoms of deep vein thrombosis and pulmonary embolism and treat as medically appropriate.

8.4.8 Dose Modifications for Venous Thromboembolic Events

CTCAE Grade	**Abemaciclib Dose Modifications**
Grade 1 or 2	No dose modification is required.
Grade 3 or 4	Suspend dose and treat as clinically indicated. Abemaciclib may be resumed when the patient is clinically stable.

8.4.9 Dose Modifications for Other Adverse Events Not Specified in Above Tables

CTCAE Grade	**Abemaciclib Dose Modifications**
Grade 1 or 2	No dose modification is required.
Persistent or recurrent Grade 2 AE that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Omit dose until AE resolves to baseline or Grade 1 for up to 7 days. Resume at next lower dose.
Grade 3 or 4	

8.4.10 Guidelines for Pregnancy and Nursing

Abemaciclib can cause fetal harm when administered to a pregnant person. In animal studies, abemaciclib was teratogenic and caused decreased fetal weight at maternal exposures that were similar to human clinical exposure based on the area under the plasma concentration versus time curve (AUC) at the recommended human dose. Therefore, teratogenicity is considered an important potential risk for abemaciclib. There are no available human data informing the drug-associated risk. Advise pregnant persons of the potential risk to a fetus. Additionally, there are no available data on effects of breastfeeding. Advise a nursing person to discontinue breastfeeding during treatment with abemaciclib.

- Persons of childbearing potential must have a negative serum pregnancy test within 7 days of the first dose of abemaciclib and agree to use a highly effective contraception method during the treatment period and for 3 weeks following the last dose of abemaciclib.
- Contraceptive methods may include an intrauterine device [IUD] or barrier method. If condoms are used as a barrier method, a spermicidal agent should be added as a double barrier protection.
- Cases of pregnancy that occur during maternal exposures to abemaciclib should be reported. If a patient is discovered to be pregnant following abemaciclib initiation, that person must discontinue treatment immediately. Data on fetal outcome and breast-feeding are to be collected for regulatory reporting and drug safety evaluation.

9. DRUG FORMULATION/STORAGE/SUPPLY

9.1 Abemacliclib (Verzenio®, LY2835219, NSC 783671)

Abemaciclib is an investigational agent and will be supplied free-of-charge from Eli Lilly and Company.

9.1.1 Background:

Abemaciclib is a selective and potent small-molecule CDK4 and CDK6 dual inhibitor.

9.1.2 Formulation:

Abemaciclib will be provided as 50 mg tablets.

9.1.3 Preparation and storage

Abemaciclib must be stored at room temperature. For specific storage instructions refer to the product label.

9.1.4 Administration

Take abemaciclib with food at approximately the same times every day. Swallow tablets whole and do not chew, crush, or split tablets before swallowing. Do not take tablets if broken, cracked, or otherwise not intact. If a patient misses or vomits a dose, take the next dose at its scheduled time.

9.1.5 Pharmacokinetic information

9.1.5.1 Absorption

Abemaciclib absorption is slow, with a median Tmax of 8.0 hours. In the therapeutic dose range of 50-200 mg, the increase in plasma exposure (AUC) and Cmax is dose proportional. Steady state was achieved within 5 days following repeated BID dosing, and abemaciclib accumulated with a geometric mean accumulation ratio of 3.3 (80% CV) and 4.6 (88% CV) based on Cmax and AUC, respectively.

9.1.5.2 Bioavailability

The absolute bioavailability of abemaciclib is 45% (90% CI: 40-51%).

9.1.5.3 Distribution

Abemaciclib was highly bound to plasma proteins in humans (mean bound fraction was approximately 96-98%), and the binding was independent of concentration from 152 to 5066 ng/mL. Abemaciclib binds to both human serum albumin and alpha-1-acid glycoprotein. The geometric mean systemic volume of distribution is approximately 747 L (68.6% CV). In patients with advanced cancer, concentrations of abemaciclib and its active metabolites M2 and M20 in cerebrospinal fluid are comparable to unbound plasma concentrations.

9.1.5.4 Metabolism

Hepatic metabolism is the main route of clearance for abemaciclib. Abemaciclib is metabolized to several metabolites primarily by CYP3A, with formation of N-desethyl abemaciclib (M2) representing the major metabolism pathway.

Additional metabolites include hydroxyabemaciclib (M20), hydroxy-N-desethylabemaciclib (M18), and an oxidative metabolite (M1). Metabolites N-desethylabemaciclib (M2) and hydroxyabemaciclib (M20) are active with similar potency as abemaciclib.

9.1.5.5 Half-life elimination

The geometric mean hepatic clearance (CL) of abemaciclib was 21.8 L/hours (39.8% CV), and the mean plasma elimination half-life for abemaciclib in patients was 24.8 hours (52.1% CV).

9.1.5.6 Excretion

After a single oral dose of [14C]-abemaciclib, approximately 81% of the dose was excreted in feces and 3.4% excreted in urine. The majority of the dose eliminated in feces were metabolites.

9.1.5.7 Potential Drug Interactions

Abemaciclib is predominantly cleared by oxidative metabolism via CYP3A. 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 coadministered moderate (for example, ciprofloxacin) or weak (for example, ranitidine) CYP3A inhibitors. If coadministration with a strong CYP3A inhibitor is unavoidable, reduce the abemaciclib dose to 100 mg twice daily or, in the case of ketoconazole, reduce the abemaciclib dose to 50 mg twice daily. In patients who have had a dose reduction to 100 mg twice daily due to adverse reactions, further reduce the abemaciclib dose to 50 mg twice daily. Avoid grapefruit or grapefruit juice. If a CYP3A inhibitor is discontinued, increase the abemaciclib dose (after 3-5 half-lives of the inhibitor) to the dose that was used before starting the inhibitor.

9.1.6 Known potential adverse events

Most common adverse reactions (incidence ≥20%) were diarrhea, neutropenia, nausea, abdominal pain, infections, fatigue, anemia, leukopenia, decreased appetite, vomiting, headache, and thrombocytopenia.

In the MONARCH 2 study, venous thromboembolisms (VTEs) were experienced by 21 patients (4.8%) in the abemaciclib plus fulvestrant arm and by 2 patients (0.9%) in the placebo plus fulvestrant arm. In the MONARCH 3 study, VTEs were experienced by 16 patients (4.9%) in the abemaciclib plus non-steroidal aromatase inhibitor (NSAI) arm and by 1 patient (0.6%) in the placebo plus NSAI arm. In these studies, risk factors for VTEs were balanced between the study arms, and overall there was no specific risk factor identified that could be predictive of VTEs on treatment with abemaciclib.

9.1.7 Drug Ordering and Accountability

Abemaciclib will be provided free of charge by Eli Lilly & Co.

Each institution will order the drug through drug supplier. (See Pharmacy Manual for order form.)

NOTE: Local drug destruction may occur if the site provides a copy of their local drug destruction policy to the sponsor (Mayo Clinic) and the respective drug supplier (Eli Lilly & Co).

10. CORRELATIVE/SPECIAL STUDIES

10.1 Collection Tables

10.1.1 Cohort B Group 3

Specimen Type	Pre-Reg[24]	After Registration prior to treatment[25] (Baseline)	At time of surgery
Research Blood (70 mL)		X	
Research Stool		X	
Research Tissue	X (needle cores from biopsy and unstained slides or tissue blocks from archived FFPE tissue if available)		X (FFPE tissue block/slides required)

See Lab Manual for specifics of specimen collection and processing

Note: Surgical FFPE tissue block/slides (with residual tumor or tumor bed) is required at time of surgery to assess study endpoints.

Note: If patient does not have surgery, then a research biopsy should be performed and needle core biopsies obtained – follow same procedures as for Pre-Registration biopsy, although an additional clip does not need to be placed.

[24] ≤35 days prior to registration (after completion of NAC)
[25] Baseline stool kit may be collected any time after consent is obtained and pre-registration patient study ID is available. Preferably ≤14 days prior to first treatment with abemaciclib

10.1.2 Cohort B Group 4

Specimen Type	Pre-Reg[26]	After Registration prior to treatment (Baseline)	Prior to surgery	At time of surgery
Research Blood (70 mL)		X[27]	X	
Research Stool		X[28]	X	
Research Tissue	X (needle cores from biopsy and unstained slides or tissue blocks from archived FFPE tissue if available)			X (FFPE tissue block/slides required)

See Lab Manual for specifics of specimen collection and processing

Note: Surgical FFPE tissue block/slides (with residual tumor or tumor bed) is required at time of surgery to assess study endpoints.

Note: If patient does not have surgery but has received at least 14 days of treatment, then a research biopsy must be performed and needle core biopsies obtained – follow same procedures as for Pre-Registration biopsy, although an additional clip does not need to be placed.

[26] ≤35 days prior to registration (after completion of NAC)
[27] ≤14 days prior to first treatment
[28] Baseline stool collection kit may be given to patient any time after consent is obtained and pre-registration patient study ID is available. Preferably ≤14 days prior to first treatment with abemaciclib.

10.2 Blood Samples

10.2.1 Circulating Tumor Cells (CTC)

Whole blood samples will be collected to isolate circulating tumor cells. These samples will be shipped immediately according to the lab manual to Mayo Clinic Rochester for processing.

The markers used for CTC identification may include DAPI or SYTOX orange, cytokeratin, EpCAM, ER, HER2, ALDH1, and CD44 to distinguish between epithelial-like CTCs and EMT/stem-cell like CTCs. All CTCs will be assessed for vimentin expression. CD45 will be used to distinguish WBCs (CD45 positive) from CTCs (CD45 negative). CTC populations will be isolated for DNA extraction using established methods to allow for mutation analysis. See Lab Manual for collection and processing instructions

It is known that CTCs have biologic relevance in epithelial malignancies, and their relative abundance in the peripheral blood has strong correlations with disease related outcomes in early stage and advanced breast cancer[50-54]. Beyond simple enumeration, molecular profiling of these cells may provide clinically relevant predictive information to guide the selection of therapy. Deriving detailed molecular signatures for isolated CTCs may also facilitate drug discovery by assessing the markers unique to progression, such that relapsing patients may be treated for their current molecular disease.

The Mayo group has established experience detecting and isolated purified CTCs from breast cancer patients, as well as the capability to design 4- or 6-marker panels to capture specific phenotypic populations of CTCs.

10.2.2 Cell-free DNA (cfDNA)

Whole blood samples will be collected to isolate cfDNA. These specimens will be shipped immediately to Mayo Clinic Rochester according to the Lab Manual.

Samples will be stored until we are ready for DNA extraction and mutational analysis. Selected mutations identified at baseline will be verified and quantified in all cfDNA samples.

10.2.3 Pharmacogenomics

Whole blood sample will be collected for DNA and proteomics. These specimens will be shipped immediately to Mayo Clinic Rochester according to the Lab Manual.

Pharmacogenomics is the study of the role of genetic variation in the individual variation of drug response and toxicity. In the specialty of oncology, drug response phenotypes vary based upon both tumor (somatic), as well as host (germ-line) genetic variation. Our aims are to identify single nucleotide polymorphisms (SNPs) or genetic variants associated with treatment resistance and development of adverse drug effects. DNA will be genotyped (or sequenced) based on the most up to date technology. Current technology platforms such as the Illumina OmniExpress BeadChip (San Diego, CA, USA) are often used for GWAS studies but newer platforms will be used at the time of genotyping/sequencing. Sample quality control will be carried out by methods including identity-by-state to evaluate cryptic

relatedness for each sample and population stratification by the use of principal component analysis to exclude genetically heterogeneous samples from further analysis.

10.2.4 Peripheral blood immune phenotype analyses with CyTOF

Whole blood sample will be collected for PMBCs according to the lab manual.

Analyses of tumor-host immune interaction studies have largely focused on exploring the interplay between cancer cells and immune cells at the tumor tissue level. Analyses evaluating the tumor microenvironment have identified that the robustness of immune cell infiltration in TNBC is both prognostic and predictive of response to chemotherapy and immunotherapy. We will aim to evaluate whether such changes in CD8+ T cells, regulatory T cells, and other immune cell populations are detectable beyond the tumor microenvironment, in peripheral blood. To this end, we will leverage multiplexed technologies such as Cytometry by Time-of-Flight (CyTOF^{TM}), which have greatly improved the ability to evaluate a large number of surface markers at the single cell level, allowing for deep characterization of circulating immune cell populations in peripheral blood specimens.

Here, we will test the hypothesis that treatment with abemaciclib will be associated with expansion of the peripheral blood CD8+ T cell compartment, and contraction of the peripheral blood Treg compartment, mirroring what has been observed in tissues preclinically.

10.3 Stool Samples

10.3.1 Research Stool Collection

Timepoints:

- Pre-Registration –stool kit provided at pre-registration (All patients)
- Prior to surgery–stool kit provided prior to surgery Cohort B Group 4 only)

Samples will be collected and processed per lab manual and used for multi-omic analysis to evaluate changes in the microbiome with exposure to abemaciclib.

There is growing evidence that the large number of residential microbes (e.g. viruses, bacteria, eukaryotes, others) that colonize the human gastrointestinal tract have fundamental roles in health and disease (Kwa M, 2016). It has recently emerged that the gastrointestinal microbiome can influence cancer development and therapeutic response. For example, studies have shown that the composition of gut bacteria can influence the therapeutic efficacy of immune checkpoint inhibitors, and that therapeutic efficacy can be improved through its modulation (Gopalakrishnan V, 2018) (Routy B, 2018). Further, there is evidence that variations in the composition and activities of enteric bacterial genes that metabolize estrogen may influence the development of postmenopausal HR-positive breast cancer. The development of a criterion based not only on the genetic makeup of a tumor biopsy, but also on the gut microbiome and other biomolecular features (e.g. serum metabolites, cytokine profiling) that can determine whether a particular drug may be effective has garnered interest in personalized medicine (Chen R, 2013).

10.4 Tissue Samples

10.4.1 Research Biopsy Collection

Timepoints:

- with clip placement during Pre-Registration (all patients); and
- End of abemaciclib, if surgery will not be performed (Cohort A Group 2 and Cohort B Group 4 only)

Ultrasound-guided biopsy to obtain 6-8 tissue cores using 14 gauge needle.

If no target seen on ultrasound, then stereotactic biopsy should be performed obtaining 4-6 tissue cores using 9 gauge needle.

First and second tissue cores will be submitted for routine FFPE processing.

A section from each of the first two cores will be evaluated by H&E for patient eligibility. The H&Es, corresponding FFPE tissue blocks (with residual core 1 tissue and residual core 2 tissue) will be sent to Mayo Clinic in Rochester, MN, for research purposes.

The remaining tissue cores will be snap frozen (with the exception of cores 5 and 6 at Mayo Rochester only which will be processed for PDX). Please see Lab Manual for specimen processing details and core prioritization.

Additional FFPE derived tissue sections will be used to evaluate the effects of abemaciclib on the immune phenotype of FFPE tumor sections will be processed to examine the following:

- genes directly involved in tumor cell antigen presentation (e.g., B2M, HLA-A, HLA-B, HLA-C, TAP1, TAP2, TAPBP)
- interferon-stimulated genes (ISGs) that regulate antigen presentation (e.g., STAT1, NLRC5) and other ISGs (e.g., IRFs, OAS2)
- genes involved in dsRNA response (e.g., DDX58, DHX58)
- genes encoding interferons, including type 3 IFNs (e.g., IFNL1, IFNL2, IFNL3)
- genes indicating a cytotoxic T cell response (e.g., PRF1, GZMB)
- Treg-specific transcription factor genes (e.g., FOXP3, IKZF2).

DNA and RNA extraction will be performed and sequencing will be completed.

Baseline research biopsy, will require hematoxylin and eosin (H & E) staining to be performed on the first and second cores obtained as part of standard of care procedure. Additional analyses will be performed on remaining tissue at a later date.

10.4.2 Surgical Tissue Collection

At the time of surgery the surgical specimen will be processed according to Mayo Clinic Pathology Lab standards. The pathologist should ensure the identification of the appropriate clip denoting the research biopsy site, and collect and submit residual tumoral tissue or tumor bed around the research biopsy clip to ensure evaluation of tissue in the same region as the research biopsy. The surgical tissue will be prioritized according to research needs or best utilization as indicated in the lab manual.

In cases where there is no residual invasive cancer in the breast, a sample of the residual tumor bed adjacent to the research biopsy site (denoted by a clip – as above) should be submitted according to the lab manual.

In cases with residual disease in the lymph nodes (sentinel lymph node and/or any other axillary lymph nodes), then tissue from grossly positive lymph nodes will be flash frozen.

In the case where there is multifocal disease remaining in the breast at the time of surgery tissues from each of the residual tumors will be submitted for processing (following the above protocol).

For patients with bilateral disease the tissue from the index side will be processed as outlined above and any residual disease from the contralateral side will be processed per usual pathology guidelines and not submitted.

10.4.3 PDX (Mayo Clinic Rochester Patients Only)

Xenografts will be generated in Dr. Liewei Wang's lab (Mayo Clinic). Her lab has already successfully generated a series of breast cancer models for all subtypes from both primary breast cancer as well as metastatic breast cancer using core biopsy or surgical samples. In this study, to generate individual tumor xenograft line for drug screening, drug cytotoxicity assay, specimen obtained during the biopsy will be immediately injected into NGS mice for the creation of human tissue xenografts (see IACUC A49111 and IACUC A00003279-17). The xenograft tumor samples will be used to determine the functional implications of tumor alterations identified from tumor sequencing and for future drug screening and cytotoxicity assay. These lines will be also useful for future research purposes, such as testing novel compounds and different regimens. All the tumor tissues from xenografts will be stored on Gonda 19-466E, Mayo Clinic, 200 First St SW, Rochester MN 55905.

10.4.4 Organoids (all sites)

Organoids will be generated in Dr. Liewei Wang's lab (Mayo Clinic, Rochester, MN)

10.4.5 Drug Screen (*ex vivo*)

Tumor tissue will be sent for drug screening using a 3D microcancer model to identify potential targets which could also be validated in the PDX created at progression. Results will be interpreted in the context of sequencing data from ctDNA and/or tissue collected after abemaciclib administration.

10.4.6 Microcancers: 3D models of solid tumors for drug sensitivity screening

Validation of companion therapeutic biomarkers for personalized therapy of solid tumors has been slow because of the lack of appropriate model systems to test biomarker-guided therapies. In the past, conventional cell lines have been used extensively with little success. It is now believed that their forced adaptation to two-dimensional (2D) culture conditions leads to loss of key genetic and functional characteristics and gain of others, making them unreliable models of human cancer. Conversely, genetically engineered animal cancer models rarely recapitulate human cancer in a personalized manner, while models based on human cancer xenografts in immunocompromised mice take months to develop. The advent of three-dimensional

(3D) tissue modeling in vitro, is a promising mechanism to fill the gap and to provide reliable, sensitive, and affordable characterization of tumor samples for biomarker validation and response to treatment.

Multiple studies have shown that human cancer cells extracted fresh from solid tumors can grow in mice or in 3D culture in a superior manner to growth in 2D culture for drug sensitivity studies. Likely explanations for this are the presence of both cancer cells and tumor microenvironment in a more physiological 3D context, and the overreliance of cells grown in 2D culture to tension-induced signaling, otherwise known as mechanotransduction, for their growth in plastic. Interestingly, growth of normal human tissues in 3D culture has been achieved for many tissues, under conditions that preserve the stem cell compartment and retain the normal tissue architecture.

3D model systems have several advantages over animal models. A special consideration is that all proliferative models of drug screening, including 2D, 3D organoids, and PDX models, are subject to selective pressure and tumor evolution. The tumor microenvironment is increasingly recognized as a key contributor to cancer progression and resistance to therapy. Therefore, removing selective pressure by optimizing culture conditions, and maintaining the tumor microenvironment are essential for accurate prediction of response to treatment.

The laboratory of Dr. Panagiotis Anastasiadis has developed a non-proliferative 3D microcancer model process that minimizes selective pressure and maintains the microenvironment. This model provides the ability to maintain individual patients' tumors in short-term 3D culture, in a manner that allows both biomarker validation and drug screening in a timely and sensitive manner. The Anastasiadis lab has developed and optimized the process and has successfully grown every patient tissue received to date, including tumors of the pancreas, liver, breast, brain, kidney, ovary and bladder. They have optimized 96-well based cytotoxicity assays and established assay sensitivity to as few as 500 cells, allowing the rapid interrogation of potential biomarkers and drug treatments in single patient biopsies.

Dr. Liewei Wang will utilize Dr. Anastasiadis' established methods.

The drug screening panel will include FDA approved regimens selected for BEAUTY-2 (MC1734).

10.4.7 JAK2

Triple negative breast cancer (TNBC) is an aggressive disease with poor clinical outcomes. Thus there is a need to identify markers that can be exploited for improved clinical care. We and subsequently others have identified amplification of chromosome 9p24.1 encoding PD-L1, PD-L2, and JAK2 (the PDJ amplicon) as a clinically relevant driver of aggressive TNBC. Furthermore there is emerging data suggesting that the presence of the amplicon is associated with increased JAK2/pSTA3 signaling and a reprogrammed immune environment. Recent studies have demonstrated that presence of PDJ amplifications is associated with response to checkpoint inhibitors (ICI). Studies have shown that PDJ can be induced with chemotherapy. In order to determine whether this is a biomarker of immune

dysregulation and an inducible biomarker we will perform JAK2 FISH analysis on the pretreatment biopsies and biopsies on patients with residual disease. The requirement will be 2 unstained slides (5 um sections) with one scanned H&E slide with the area of interested circled. Analysis of JAK2 amplification will be performed in the Immunohistochemistry Core at the Mayo Clinic in Rochester, MN.

10.5 Genetic Testing

Participants will be given information as part of the informed consent process that samples will be used for research tests that will include genetic studies and testing. The intent is not to give participants (or his/her medical providers) the results of any testing done for research purposes; however, incidental germline (heritable) mutations may be identified of which a participant may or may not already be aware. In the case that an incidental genetic finding is identified, the Protocol Chair of this project will be notified. The possible decisions for handling incidental findings may include notification of the participant (and provider); recommendation for genetic counseling, which may or may not include genetic testing (e.g., if the finding was not done in a CLIA certified laboratory); or, neither. In general, a member of the participant's treating team will be given the information to help with notification. In all cases, the current policy of the Mayo Clinic and local/participating site IRB, as applicable, will be followed and any additional approvals that may be required prior to participant notification will be secured in advance.

10.6 Additional Information

Submission of data for Genome Wide Association Studies (GWAS) is not currently planned; however, subjects will be asked for permission in the informed consent process. A revision to the protocol and/or any regulatory approvals will be secured prior to any GWAS submission or inclusions in the future, if applicable.

11. SPECIMEN BANKING

The study Protocol Chair and collaborators have approval to use all research biospecimens collected during the conduct of this trial to address the research questions described in the protocol document. All future use of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by Mayo Clinic Cancer Center according to its established policies, whether the specimens are stored in a central site or at a local institution in a virtual repository.

Secondary use of biospecimens for new endpoints must be submitted to the study chairs for possible reviewe.

12. MEASUREMENT OF EFFECT: COHORT A GROUP 2 AND COHORT B GROUP 4

CD8/FOXP3 protein expression will be determined in tumor tissue obtained by image guided percutaneous breast biopsy after completion of NAC (prior to abemaciclib) and in residual invasive breast cancer obtained at surgery or post-treatment biopsy (after completion of 14-21 days of abemaciclib).

13. ADVERSE EVENT REPORTING REQUIREMENTS

13.1 General

Adverse event collection and reporting is a routine part of every clinical trial. This study will use the descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events current version (CTCAE v5.0) that is available at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Information on all adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Adverse events experienced by participants will be collected and reported from initiation of study medication, throughout the study, and within 30 days of the last dose of study medication. Participants who experience an ongoing adverse event related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

NOTE: All sites must notify Study Chair within twenty-four (24) hours of Investigator and/or Institution receiving notification of any “serious” adverse event experienced by a patient participating in the Study and receiving Study Drug. Study Chair will review and provide approval to forward to Lilly within 2 days of notification.

Investigator and Institution further agree to make available promptly to Lilly such records as may be necessary and pertinent for Lilly to further investigate an adverse event in the Study that is possibly associated with the Study Drug.

13.2 Definitions

13.2.1 Adverse Event (AE)

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

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

13.2.2 Serious adverse event (SAE)

A serious adverse event is an undesirable sign, symptom, or medical condition which:

- is fatal;
- is life-threatening;
- requires or prolongs inpatient hospitalization for ≥24 hours;

- results in persistent or significant disability/incapacity to conduct normal life functions;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above;
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

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

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen, or that is required per protocol
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care
- Death due to disease progression unless attributable to the study drug(s)
- A hospitalization due to an expected adverse event (e.g., hospitalization due to expected febrile neutropenia).

13.2.3 Expectedness

- Expected: Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- Unexpected: An adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk

13.2.4 Attribution

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

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

13.3 Reporting Procedures

All adverse events will be captured on the appropriate study-specific case report forms (CRFs).

13.3.1 Summary of Event Reporting for this Trial

WHO:	WHAT form:	WHERE to send:
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56 AND attach Pregnancy Reporting http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf	Send a copy to Lilly: Fax 866-644-1697 or: 317-453-3402 NOTE: MCCC form will automatically be sent to CANCERCROSAFETYIN@mayo.edu and RSTP2CSAES@mayo.edu
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56 AND attach MedWatch 3500A: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf	Submit a copy to Lilly: Fax 866-644-1697 or: 317-453-3402 NOTE: MCCC form will automatically be sent to CANCERCROSAFETYIN@mayo.edu and RSTP2CSAES@mayo.edu

13.3.2 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 13.3.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

*Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be reported in an expedited manner[1]
General disorders and administrations site conditions	Fatigue	≤Grade 3
	Malaise	≤Grade 3
Skin and subcutaneous tissue disorders	Alopecia	≤Grade 4

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- ● Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.

13.3.3 Serious Adverse Events

All serious adverse events, regardless of causality to study drug, will be reported to the Principal Investigator and the Study Coordinator at each institution, and also to the Coordinating Center.

All serious adverse events must be reported to the Coordinating Center within 1 business day after the investigator becomes aware of the event. Events should be reported using a MedWatch form (3500A) as available on the FDA website (see link below).

Follow-up information must also be reported within 1 business day of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by the Protocol Chair (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

13.3.3.1 General reporting instructions

The Mayo IND and/or MCCC Compliance will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

13.3.3.1.1 Mayo Clinic Sites

Mayo sites will use Mayo Expedited Event Report form http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56 for investigational agents or commercial/investigational agents on the same arm.

Attach the MedWatch 3500A form to the Mayo Expedited Event Report form http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56.

13.3.4 Expedited Reporting Requirements for IND Agents

13.3.4.1 Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour 3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 13.3.2 for Exceptions to Expedited Reporting

13.3.5 Other Required Reporting

13.3.5.1 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the appropriate documentation and use the Mayo Clinic Cancer Center Expedited Event Report form http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56, to submit to CANCERCROSAFETYIN@mayo.edu. The Mayo Clinic Compliance Unit will review and process the submission to the Mayo Clinic IRB and work with the IND Coordinator for submission to FDA.

13.3.5.2 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death not otherwise specified (NOS): A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

13.3.5.3 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND will be reported. Three options are available to describe the event:
  - 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 in each protocol.

13.3.5.4 Second Malignancy

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 ONLY routine reporting unless otherwise specified.

13.3.5.5 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The

patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf

13.3.5.5.1 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

13.3.5.5.2 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

13.3.5.5.3 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

13.3.6 Required Routine Reporting - Cohort B Group 4 ONLY

13.3.6.1 Baseline and Follow-up Adverse Events Evaluations

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v5.0.

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Prior to Surgery	End of Study
Blood and lymphatic system disorders	Anemia	X	X	
Gastrointestinal disorders	Baseline # of stools	X		
	Diarrhea		X	X
	Nausea	X	X	X
Infections and infestations	Wound infection			X
Injury, poisoning and procedural complications	Seroma			X
	Wound complication			X
Investigations	Alanine aminotransferase increased	X	X	
	Blood bilirubin increased	X	X	
	Aspartate aminotransferase increased	X	X	
	Neutrophil count decreased	X	X	
	Platelet count decreased	X	X	
Vascular disorders	Hematoma			X
	Thromboembolic event		X	X

13.3.6.2 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 13.3.6.1:

13.3.6.2.1 Grade 2 AEs deemed possibly, probably, or definitely related to the study treatment or procedure.

13.3.6.2.2 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

13.3.6.2.3 Grade 5 AEs (Deaths)

13.3.6.2.3.1 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

13.3.6.2.3.2 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

13.3.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

13.3.8 Institutional Review Board

All adverse events and serious adverse events will be reported to the Mayo Clinic IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to Mayo Clinic as soon as possible.

13.3.9 Food and Drug Administration (FDA)

Mayo Clinic Cancer Center Compliance has been designated to manage the Investigational New Drug Application (IND) associated with this protocol on behalf of the sponsor-investigator. Mayo Clinic Cancer Center Compliance will cross-reference this submission to Eli Lilly and Company's parent IND at the time of submission. Additionally, Mayo Clinic Cancer Center Compliance will submit a copy of these documents to Eli Lilly and Company at the time of submission to FDA.

Sponsor-Investigator will be responsible for all communication with the FDA in accordance with 21CFR312, which includes but is not limited to the 7 and 15 Day Reports, as well as an Annual Progress Report. Additionally, Mayo Clinic Cancer Center Compliance will submit a copy of these reports to Eli Lilly and Company at the time of submission to FDA.

In this trial, unexpected adverse events believed to be definitely, probably, or possibly related to the medications will be reported to the Food and Drug Administration via MedWatch. The Coordinating Center will be responsible for correspondence regarding adverse events with the FDA for all participating sites. Sites will be instructed in the method by which to report events to the Coordinating Center and per what forms (i.e., mandatory MedWatch 3500A forms available at: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf

13.3.10 Other

All sites will report SAEs as stipulated by Eli Lilly and Company (i.e., all SAEs, only those related, etc.) within **1 business day** of receipt of the SAE Reporting Form. Follow-up information will be provided to Eli Lilly and Company as reasonably requested.

14. DATA MONITORING

14.1 Monitoring

A data and safety monitoring plan (DSMP) will be conducted under the auspices of the lead/primary institution, Mayo Clinic.

Data monitoring including on-site audits will be conducted under the auspices of the lead/primary institution, Mayo Clinic, and its assignees.

15. REGULATORY CONSIDERATIONS

15.1 Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Mayo Clinic Institutional Review Board (IRB) per the current institutional standards.

Any changes to the protocol will be made in the form of an amendment and must be approved by the Mayo Clinic IRB prior to implementation.

The Protocol Chairs (or their designee) is responsible for the coordination and development of all protocol amendments, and will disseminate this information to the participating centers.

15.2 Informed Consent

The investigator (or his/her designee) will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The subject should read and consider the statement before signing and dating it, and will be given a copy of the document. No subject will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

15.3 Ethics and GCP

This study will be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

15.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

16. STUDY CONDUCT GUIDELINES

16.1 Study Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation. The required documents include, but are not limited to the following:local Delegation of Authority (DOA), financial disclosures, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. It is the responsibility of the participating sites to maintain copies of all documentation submitted.

16.2 Records Retention

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location.

16.3 Publication

It is understood that any manuscript or releases resulting from the collaborative research must be approved by the Protocol Chair and will be circulated to applicable participating sites/investigators prior to submission for publication or presentation.

Additionally, any publication of study data and results must conform to the Mayo Clinic Cancer Center publications policy.

17. STATISTICAL CONSIDERATIONS

17.1 Background

Miyashita et al demonstrated among women with triple negative breast cancer who received neo-adjuvant chemotherapy that recurrence-free survival was significant longer for those women with a CD8/FOXP3 ratio ≥1.6 in their residual tumors than those women with a CD8/FOXP3 ratio <1.6 in their residual tumors (HRadj =2.07; 95%CI: 1.03-4.44). When CD8/FOXP3 ratio was considered on a continuous scale after adjusting for other prognositic factors, it was not found to be significantly associated with RFS (reference: Breast Cancer Res 2015; 16(1):124)

Karihtala et al found that approximately 64% of primary triple negative breast cancers express vimentin and vimentin expression is associated with both high proliferation rates and high tumor grade. Unpublished Mayo Clinic data evaluating staining of vimentin in a cohort of 30 TNBC patients treated with AC followed by paclitaxel who had residual disease demonstrated a similar frequency of vimentin expression (around 60%).

Data from the NeoPalAna study (Ma et al) of women receiving neoadjuvant palbociclib with anastrozole for ER+ breast cancer reported that those women who did not have surgery the day after their last dose of palbociclib (median 29 days from the last dose of palbociclib) exhibited significantly higher Ki-67 at surgery compared to patients who went to surgery immediately after the last dose of palbociclib.[49]

As such, assessment of the primary endpoint will include all eligible women with a pre-abemaciclib CD8/FOXP3 ratio < 1.6 who completed 14-21 days of abemaciclib and underwent surgery or breast biopsy at most 3 days after last dose of abemaciclib (referred to as evaluable patients).

17.2 Study Design

The primary aim of this clinical trial is to examine the effect of abemaciclib on the CD8/FOXP3 ratio in two patient populations, namely, patients with chemotherapy resistant TNBC following neoadjuvant chemotherapy (Cohort A) and patients with patients with chemotherapy resistant TNBC following neoadjuvant chemotherapy with pembrolizumab (Cohort B).

Specifically, for each cohort, we seek to estimate the proportion of patients with a pre-abemaciclib CD8/FOXP3 ratio < 1.6 who completed 14-21 days of abemaciclib and underwent surgery or breast biopsy at most 3 days after last dose of abemaciclib who have a post-abemaciclib CD8/FOXP3 ratio ≥1.6 in their residual tumors

For each cohort, a Simon optimum two stage phase II clinical trial design was chosen to test the null hypothesis that the rate of conversion to post-abemaciclib CD8/FOXP3 ratio of 1.6 or more is ≤5% against the alternative that rate of conversion to post-abemaciclib CD8/FOXP3 ratio of 1.6 or more is ≥20% with the target probability of a type I error and probability of a type II error set to 0.10. The probability of terminating after the first stage is 0.736, if the null hypothesis is true.

Stage 1: Twenty evaluable patients will be enrolled and then registration will be temporarily halted until there are sufficient post-abemaciclib CD8/FOXP3 ratio results to evaluate the decision rule.

- If at most 1 of these 20 patients is found to have a post-abemaciclib CD8/FOXP3 ratio ≥1.6, then enrollment will be closed and a short course of abemaciclib following NAC will not be considered promising in increasing post-NAC CD8/FOXP3 ratios above the threshold of 1.6 in this patient population.
- If at 2 or more of these 20 patients is found to have a post-abemaciclib CD8/FOXP3 ratio ≥1.6, then enrollment will reopen to obtain additional 15 patients.

Stage 2: Fifteen patients will be enrolled with a post-NAC CD8/FOXP3 ratio <1.6.

- If at most 3 of these 35 patients are found to have a post-abemaciclib CD8/FOXP3 ratio ≥1.6, then a short course of abemaciclib following NAC will not be considered promising in increasing post-NAC CD8/FOXP3 ratios above the threshold of 1.6 in this patient population.
- If 4 or more of these 35 patients are found to have a post-abemaciclib CD8/FOXP3 ratio ≥1.6, then this approach will be recommended for further testing.

Note: Post-NAC CD8/FOXP3 ratios will not be known at the time of registration. Miyashita et al reported that at 50% of patients with TNBC will have a post-NAC CD8/FOXP3 ratio <1.6. As such we will begin by registering as many as 40 patients onto the first stage of the trial to obtain 20 patients with a post-NAC CD8/FOXP3 ratio <1.6. This assumption will be monitored throughout the first stage of the enrollment period. A protocol amendment will be drafted for DSMB and IRB approval if this assumption was too pessimistic or optimistic. If the assumption that 50% of the patients enrolled will have a post-NAC CD8/FOXP3 ratio <1.6 and there is sufficient evidence of activity from the first stage to open the trial to the second stage, 30 patients will be enrolled to obtain 15 patients with a post-NAC CD8/FOXP3 ratio <1.6.

A 90% confidence interval for this rate of conversion will be constructed using the Duffy-Santner approach taking into account the sequential nature of the study design.

As of Amendment 6, enrollment to Cohort A was closed related to a change in the clinical practice with neoadjuvant pembrolizumab now standardly utilized in combination with chemotherapy. At the time of closure to Cohort A, 10 patients had been enrolled. As such, a 90% binomial confidence for the rate of conversion among the Cohort A patients will be constructed.

17.3 Secondary Endpoints and analysis plans for each cohort independently

17.3.1 Adverse events

All patients who begin abemaciclib treatment will be included in the summary of adverse events.

Prior to and at discontinuation of abemaciclib treatment, adverse events will be monitored and graded with attribution assigned using CTCAE version 5.0. For each type of adverse event (AE) reported, the proportion of patients experiencing a Grade 2 or worse level of that AE will be determined.

17.3.2 Changes in Vimentin expression

Based on findings of Karihtala et al. and the Mayo team, approximately 60% of the patients enrolled are expected to have post NAC vimentin expressing invasive cancer cells. Vimentin expression will be evaluated by IHC with using 0, 1+, 2+ or 3+ scoring scheme.

A point estimate of the proportion of eligible patients whose post-NAC residual tumor Vimentin expression levels were 2+ or 3+ and then fell to 0 or 1+ after abemaciclib among the eligible patients whose began abemaciclib treatment after a post NAC residual tumor Vimentin expression level finding of 2+ or 3+ will be calculated. A 90% confidence interval for this proportion will be constructed using one-sample binomial confidence for proportions.

17.3.3 Exploration of impact of length of treatment

Association between amount of abemaciclib received and the change in CD8/FOXP3 ratio after abemaciclib will be explored graphically. A plot of the change in change in CD8/FOXP3 ratio after abemaciclib and days of treatment will be constructed to visually assess for trends.

Also, the a 90% confidence interval for the difference in binomial proportions will be constructed to assess whether the proportion of patients whose post abemaciclib CD8/FOXP3 ratio ≥1.6 after completing 14-21 days of abemaciclib differs between those who discontinued abemaciclib the day prior to surgery and who discontinued abemaciclib 2 or more days prior to surgery

A 90% binomial confidence interval for the proportion of women who failed to complete 14-21 days of abemaciclib or underwent surgery or breast biopsy 2 or more days after last dose of abemaciclib among the eligible women who began abemaciclib treatment.

17.4 Correlative Endpoints for each cohort independently

Changes in mitoses, nuclear pleomorphism, and tubule formation, tumor Ki-67, serum TKI, SNAIL, SLUG, E-Cadherin, and tumor-infiltrating lymphocytes may be examined graphically overall and by TNBC subtypes.

17.5 Exploratory objectives for each cohort independently

Effect of abemaciclib on tumor RNA sequencing followed by standard and customized pathway and enrichment analysis (e.g., Gene set enrichment analysis (GSEA) and CIBERSORT methods). The GSEA method determines whether an a priori set of genes are over-represented in the differential expression analysis of the pre- and post- drug response phenotypes. Similarly, CIBERSORT estimates cell composition from RNA transcriptomic data (e.g. immune cell populations).

The effects of abemaciclib on FFPE tumor sections (imaging mass cytometry multiplex quantitative RNA/protein immune-based profilings, such as Hyperion TM, Nanostring GeoMX, Codex (all available at Mayo Clinic)) which may include examination of:

- genes directly involved in tumor cell antigen presentation (e.g. B2M, HLA-A, HLA-B, HLA-C, TAP1, TAP2, TAPBP)
- interferon-stimulated genes (ISGs) that regulate antigen presentation (e.g. STAT1, NLRC5) and other ISGs (e.g. IRFs, OAS2)
- genes involved in dsRNA response (e.g. DDX58, DHX58)
- genes encoding interferons, including type 3 IFNs (e.g. IFNL1, IFNL2, IFNL3)
- genes indicating a cytotoxic T cell response (e.g. PRF1, GZMB)
- Treg-specific transcription factor genes (e.g., FOXP3, IKZF2).

The effects of abemaciclib on the peripheral blood immune phenotype will be assessed using mass cytometry (e.g,. CyTOF) to analyze a panel of 29 cell surface markers optimized for identification of human immune cell subsets, including the following targets: CD45, CD195 (CCR6), CD19, CD127, CD38, IgD, CD11c, CD16, CD194 (CCR4), CD123/IL-3R, TCRgd, CD185 (CXCR5), CD3, CD45RA, CD27, CD29, CD66b, CD183 (CXCR3), CD161, CD45RO, CD197 (CCR7), CD8a, CD25/IL-2R, CD20, HLA-DR, CD4, CD14, CD56/NCAM. Identification of specific immune cell subsets will be assessed using consensus clustering methods of pre-defined combination of markers.

The collection of JAK-2 will be used to assess the difference in the frequency of JAK-2 amplification among patients whose post-abemaciclib CD8/FOXP3 ratio ≥1.6 and that among patients whose post-abemaciclib CD8/FOXP3 ratio <1.6. A 95% binomial confidence for the difference in two independent proportions will be constructed for the difference in the percentage of patients with a post-abemaciclib CD8/FOXP3 ratio ≥1.6 among those with JAK-2 amplified TNBC and those with JAK-2 non-amplified TNBC.

The collection of microbiome information via stool collection will be used to generate descriptive analyses about the study population, as the total number of samples is expected to be insufficient for full statistical analysis. We will explore putative mechanistic connections underlying bacteria-drug interactions in all patients and attempt to identify the biomolecular features within the gut (stool) microbiome and its association with the pharmacokinetics and pharmacodynamics of abemaciclib.

17.6 Accrual and study duration

With the closure of Cohort A, we plan to continue to pre-register 104 TNBC patients who completed an NAC regimen that included pembrolizumab. Those patients with evidence of residual disease on imaging are eligible to register onto the study. wWe anticipate that 25 patients will be found to have no invasive disease in their biopsy specimen and will go directly to surgery.

Of the remaining 78 patients with residual invasive disease who register to the trial and begin abemaciclib, we anticipate that 50% of these patients will have a pre-abemaciclib a CD8/FOXP3 ratio ≥1.6 and another 5% will fail to complete 14 to 21 days of abemaciclib within 3 days of surgery, leaving 35 evaluable patients.

We anticipate that the rate of patient registration will be 2-3 patients per month. Accrual period will be approximately 30 months. The analysis of the primary endpoint is expected to be completed approximately 6 months thereafter.

17.7 Safety monitoring

The study chair and study statistician will review the trial data every 3 months to identify accrual, adverse events, and feasibility isuse that might be developing. The study statistician will prepare a report containing accrual, adverse events (including whether the safety threshold has been crossed) and study conduct issues which will be submitted to the MCCC Data and Safety Monitoring Board on a semi-annual basis.

The safety threshold will be examined utilizing all eligible patients who started protocol treatment.

17.7.1 Safety Threshold for each cohort

If 3 or more of the first 10 patients enrolled or 30% of more of patients enrolled thereafter develops a Grade 4 adverse event (AE) possibly, probably or definitely related to treatment, the enrollment to the trial will be temporarily suspended so that all AE data can be examined. The study chair and the study statistician will formulate a trial recommendation to present to the MCCC DSMB for approval.

17.8 Inclusion of Women and Minorities

This study will be available to all eligible patients regardless of race or ethnic group. The expected number of patients per racial/ethnicity categories are presented in the following table. The sample size for this trial was not increased in order to provide additional power for analyses by race or ethnicity.

NOTE: We have excluded men from enrollment in this trial for two reasons:
1) Breast cancer is not common in males so only one or two patients are likely to be enrolled; and
2) Because this trial is small, the addition of one or two male patients may confound the results without providing meaningful data.

Accrual Targets for Cohort B ONLY			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	11		
Not Hispanic or Latino	93		
Ethnic Category: Total of all subjects	104	0	104
Racial Category			
American Indian or Alaskan Native	0		
Asian	3		
Black or African American	3		
Native Hawaiian or other Pacific Islander	0		
White	98		
Racial Category: Total of all subjects	104	0	104

Ethnic Categories:	**Hispanic or Latino –** a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino." **Not Hispanic or Latino**
Racial Categories:	**American Indian or Alaskan Native –** a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment. **Asian –** a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.) **Black or African American –** a person having origins in any of the black racial groups of Africa. **Native Hawaiian or other Pacific Islander –** a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. **White –** a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

18. BUDGET

18.1 Costs charged to patient

Routine clinical care

18.2 Treatment and tests to be research funded

Abemaciclib, research blood work, and tumor biopsy at pre-registration (and if surgery is not done)

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APPENDICES

Appendix A: ECOG Performance Status Scale
Appendix B: Study Drug Diary
Appendix C: Lilly Guidance for Hepatic Treatment Emergent Abnormality

APPENDIX A: ECOG-Karnofsky Performance Status Scale

Grade	ECOG Definition*	Karnofsky Definition	Karnofsky Equivalent
0	Fully active, able to carry on all pre-disease performance without restriction	Asymptomatic	100
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	Symptomatic, fully ambulatory	80 – 90
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours	Symptomatic, in bed less than 50% of day	60 – 70
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.	Symptomatic, in bed more than 50% of day, but not bedridden	40 – 50
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.	Bedridden	20 – 30
5	Dead		

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From http://www.ecog.org/general/perf_stat.html

APPENDIX B: Study Drug Diary (Group 4 only)

Document removed - The study drug diary is provided as a standalone document as required by the Mayo Clinic Foundation Institutional Review Board (IRB). This version is removed to avoid the appearance of conflicting versions in the case of audit.

APPENDIX C: Lilly Guidance for Hepatic Treatment Emergent Abnormality

Liver testing including ALT, AST, alkaline phosphatase (ALP), total bilirubin (TBL), direct bilirubin (D. Bil), gamma-glutamyltransferase (GGT), and creatine kinase (CK), 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...	develops the following elevations:
ALT or AST <1.5×ULN	ALT or AST ≥5×ULN or ALT or AST ≥3×ULN concurrent with TBL ≥2×ULN
ALT or AST ≥1.5×ULN	ALT or AST ≥3× baseline or ALT or AST ≥2× baseline concurrent with TBL ≥2×ULN

If the 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 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 abuse). In addition, the evaluation should include a blood test for prothrombin time (PT-INR); serological tests for viral hepatitis A, B, C, E, 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 phosphatidylethanol. 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.

Additional Hepatic Safety Data Collection
Additional safety data (Table 8.4.3) should be collected via the CRF if 1 or more of the following conditions occur:

In participants enrolled with baseline ALT or AST <1.5×ULN

- Elevation of serum ALT or AST to ≥5×ULN on 2 or more consecutive blood tests
- The combination of elevated ALT or AST ≥3×ULN and elevated TBL ≥2×ULN

In participants enrolled with baseline ALT or AST ≥1.5×ULN

- Elevated ALT or AST ≥3× baseline on 2 or more consecutive tests
- The combination of elevated ALT or AST ≥2× baseline and elevated TBL ≥2×ULN

In all study participants

- Discontinuation from study intervention due to a hepatic event or abnormality of liver tests
- Occurrence of a hepatic event considered to be an SAE

Hepatic Monitoring Tests for a Hepatic Treatment Emergent Abnormality

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

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

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