Protocol GEICAM/2019-01

Phase II, randomized, open-label, international, multicenter study to compare efficacy of standard chemotherapy vs. letrozole plus abemaciclib as neoadjuvant therapy in HR-positive/HER2-negative high/intermediate risk breast cancer patients

"CARABELA Study"

SPONSOR:

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SUMMARY OF THE STUDY PROTOCOL

Study Title: Phase II, randomized, open-label, international, multicenter study to compare efficacy of standard chemotherapy vs. letrozole plus abemaciclib as neoadjuvant therapy in HR-positive/HER2-negative high/intermediate risk breast cancer patients "CARABELA Study"

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Indication: High/intermediate risk hormone receptor (HR) positive/human epidermal growth factor receptor 2 (HER2) negative breast cancer patients with indication of neoadjuvant therapy.

Countries and approximate number of sites: Approximately 34 sites in 2 European countries.

Number of patients: Approximately 200 evaluable patients will be enrolled in the study.

Study Rationale:

Neoadjuvant chemotherapy (NACT) was introduced in the 1970s, aiming to downstage locally advanced (inoperable) disease and make it operable. NACT was subsequently extended to operable (early) breast cancer (BC), mainly to allow breast-conserving surgery, and is now widely used, particularly for human epidermal growth factor receptor 2 positive (HER2-positive),+), triple negative (TN) and high risk estrogen receptor positive (ER-positive) and HER2- negative BC patients(1).

Neoadjuvant therapy trials offer an excellent strategy for drug development and discovery in BC(2) . A fundamental change in this paradigm happened in 2013 when the Food and Drug Administration (FDA)(3) approved the rate of pathological complete response (pCR) after NACT, that is absence of cancer in the breast and lymph nodes in the surgical specimen, as a surrogate marker of long term outcome in order to support accelerated approval. Nevertheless, the final full approval of such drugs is still dependent on demonstration of an improvement in event-free survival (EFS).

Recently, an increasing number of neoadjuvant trials are testing new drugs in combination with endocrine therapy (ET) in patients with ER-positive breast cancer(2). There are several studies evaluating the addition of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) to ET versus CT in the NA setting:

- NeoPalAna study (NCT01723774)(4): single-arm phase II trial to determine the

antiproliferative activity of palbociclib when added to anastrozole in pre- and postmenopausal women with newly diagnosed clinical stage II/II ER-positive (Allred score 6-8), HER2-negative (0 or 1+ by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) negative) BC. The primary objective was to determine whether the addition of anastrozole to palbociclib induces a higher rate of complete cell cycle arrest (CCCA: Ki67 ≤ 2.7%) than that achieved by anastrozole alone as initial therapy at C1D15. The study was designed to ensure the sample size for the PIK3CA WT cohort and the overall population for the primary endpoint analysis. Fifty patients were enrolled, 16 in the PIK3CA MUT cohort, 32 in the PIK3CA WT cohort, and 2 with unknown PIK3CA status. Thirty-nine patients completed NAT and underwent definitive breast and axillary surgery. The rates of CCCA with palbociclib plus anastrozole were significantly higher at C1D15 than that at C1D1 with anastrozole monotherapy for all evaluable patients (87% vs. 26%, P < 0.001), PIK3CA MUT (100% vs. 25%, P < 0.001), and PIK3CA WT (79%vs. 25%, P < 0.001) cohorts. There was a greater variability in Ki67 response among the PIK3CA WT tumors. Palbociclib enhanced cell-cycle control over anastrozole monotherapy regardless of luminal subtype (A vs. B) and PIK3CA status with activity observed across a broad range of clinicopathologic and mutation profiles.

- N007 study (NCT01709370)(5): The purpose of the study was to test the efficacy of neoadjuvant palbociclib therapy and to evaluate its impact on cell cycle arrest and changes in EndoPredict (EP) scores before and after treatment. Postmenopausal women with histologically proven ER-positive, HER2−negative invasive BC, ≥ 2 cm, were enrolled in an open-label, single-arm study. Twenty eligible patients were given letrozole 2.5 mg/day plus palbociclib 125 mg/day for 3 out of 4 weeks in repeated cycles for 16 weeks (4 cycles) before surgery. The primary end points were clinical response rates (cRR) and preoperative endocrine prognostic index (PEPI). Seventeen patients showed a clinical response of ≥ 50%. There was significant reduction in area (P < 0.0001) and volume (P = 0.017) of the cancer. Pathologic complete response (pCR) was achieved in 1 patient. Ki67 (P = 0.044) and EP scores (P < 0.0001) were significantly reduced after treatment. Only 8 of 20 (40%) patients had a Ki67 < 2.7 at the time of surgery after treatment. Three of 20 (15%) patients were determined to have Ki67 over 15% after treatment.
- PALLET study (NCT02296801)(6): randomized phase II study to evaluate palbociclib in addition to letrozole as neoadjuvant therapy in ER-positive early BC. Postmenopausal women with tumors ≥ 2.0 cm were randomly assigned 3:2:2:2 to letrozole 2.5 mg/day for 14 weeks (A); letrozole for 2 weeks, then palbociclib plus letrozole for 14 weeks (B); palbociclib for 2 weeks, then palbociclib plus letrozole to 14 weeks (C); or palbociclib plus letrozole for 14 weeks (D). Palbociclib 125

mg/day was administered orally on a 21-days-on, 7-days-off schedule. Co-primary endpoints for letrozole vs. palbociclib plus letrozole groups (A v B + C + D) were change in Ki67 between baseline and 14 weeks and clinical response after 14 weeks. Complete cell-cycle arrest was defined as Ki67 ≤ 2.7%. Three hundred seven patients were recruited. Clinical response was not significantly different between palbociclib plus letrozole and letrozole groups (P = 0.20; CR + PR, 54.3% vs. 49.5%). More patients on palbociclib plus letrozole achieved CCCA (90% vs. 59%; P < 0.0001).

- NeoPAL study(7) (NCT02400567): randomized, parallel, non-comparative, proof-of-concept, phase II study. Patients with ER-positive, HER2 negative, Prosigna®-defined luminal B, or luminal A and nodal status (N) positive, stage II–IIIA BC, not candidate for BCS (breast conserving surgery), were randomly assigned (1:1) to either letrozole (2.5 mg/day) and palbociclib (125 mg/day, 3 weeks on and 1 week off) during 19 weeks (4.8 months), or to 5-fluorouracil, epirubicin (100 mg/m^2) and cyclophosphamide (FEC100) followed by docetaxel 100 mg/m^2 with three 21-day courses. Sentinel lymph node (SLN) surgery was allowed only after completion of the NA therapy. One hundred and six randomized patients had a median Prosigna® score of 71 (22-93), thus leading ~85% of tumors to be classified as 'high-risk'. The interim analysis mandated the accrual to be stopped early. The primary endpoint was RCB 0–1 rate on the ITT (intent to treat) population (locally assessed according to MDACC recommendations): letrozole/palbociclib 7.7% (95% confident interval (CI) 0.4–14.9) and CT 15.7% (95% CI 5.7–25.7). This study did not reach the primary endpoint of 20% RCB 0-I after 19 weeks of treatment (7.7%; 95% CI 0.4-14.9).
- CORALLEEN study(8) (NCT03248427): a parallel-arm, multicenter, randomized, open-label, phase II clinical trial for postmenopausal women with stage I-IIIA, HR-positive, HER2-negative, luminal B according to PAM50 intrinsic subtype, BC, with tumor size ≥ 2 cm by magnetic resonance imaging (MRI). Patients were randomly assigned (1:1) to receive NACT (adryamicin/doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 (AC) for 4 cycles followed by weekly paclitaxel 80 mg/m^2 for 12 weeks) vs. six 28-day cycles of letrozole 2.5 mg/day plus ribociclib 600 mg/day for 3 weeks on, 1 week off, for 24 weeks. Primary endpoint was to evaluate the proportion of patients with PAM50 low risk of relapse (ROR) disease at surgery in the modified intention-to-treat (ITT) population including all randomly assigned patients who received study drug and had a baseline and at least one post-baseline measurement of ROR score. One hundred and six patients were enrolled. At baseline, 92 (87%) had high ROR disease and 14 (13%) patients had intermediate-ROR disease. At surgery, 23 (46·9%; 95% CI 32·5-61·7) of 49 patients in the letrozole plus ribociclib group and 24 (46·1%; 32·9-61·5) of 52

patients in the CT group were low-ROR.

- NEOLBC study(9) (NCT03283384): an ongoing randomized, multicenter, open-label, phase II clinical trial in n = 100 postmenopausal patients with HR-positive HER2-negative, stages II/III BC. Based on Ki67 levels after two weeks of initial letrozole treatment, patients are advised to continue ET with letrozole (if Ki67 level <1%) or to be randomized between standard CT [2-weekly plus G-CSF (granulocyte colony stimulating factor) followed by a taxane (3-weekly docetaxel or weekly paclitaxel)] vs. letrozole in combination with ribociclib (if Ki67 ≥1%). The aim of the study is to evaluate if CT could be replaced by the combination of letrozole plus ribociclib as a NA therapy for patients with non-metastatic primary luminal BC. The primary endpoint is to measure the difference in CCCA defined as Ki67 < 1% determined by IHC between letrozole plus ribociclib vs. CT in the surgical specimen (around seven months after starting the initial treatment with letrozole) and to determine if letrozole plus ribociclib is associated to a ≥ 100% improvement in CCCA as compared to CT in the surgical specimen.

PREDIX LumB study(10) (NCT02603679): an ongoing randomized phase II clinical trial in n=200 luminal A/B BC patients with regional lymph node metastases, comparing NA weekly paclitaxel vs. standard ET plus palbociclib for 12 weeks; after 12 weeks treatment is switched crossover. Choice of ET is for pre- and perimenopausal women and all men tamoxifen, alternatively for women in this age cohort, a luteinizing hormone-releasing hormone (LHRH) analogue in combination with an aromatase inhibitor (AI), for all postmenopausal women treatment with an AI. During the 24-weekly treatment period, clinical and radiological evaluations are performed repeatedly. Switch between the treatment groups is allowed in case of lack of response or toxicity. Postoperatively, patients receive 3-weekly courses of CT with a combination of epirubicin and cyclophosphamide (EC). The primary endpoint is Radiological Objective Response Rate after completion of the first 12-week treatment period.

RCB as the only primary endpoint is not an adequate measure to capture the CDK4/6i mechanism of action, at least not if short duration, because these are drugs that rarely induce apoptosis in BC.

These studies are optimized regarding the CT duration (4-6 months), but not for ET duration.

The optimal length of ET in the NA setting is unknown, although there are data from a sequential cohort study showing a significantly higher clinical and pathological response with longer duration: cCR (clinical complete response) of 12.5%, 42.1% and 57.7% and pCR of 2.5%, 5% and 17.5% with 4, 8 and 12 months, respectively(11).

In early BC (EBC), use of NA therapy is an attractive option to facilitate breast conservation and, critically, enables the assessment of *in vivo* biomarkers to identify proof-

of-principle activity or predict responsive or resistant subgroups of tumors.

Some ER-positive breast tumors derive limited benefit from CT and might be treated with an exclusive ET-based approach(12). In the ACOSOG Z1031B study(13) , tumors resistant to NAET [defined by Ki67 > 10% after 2-week treatment with an aromatase inhibitor (AI), anastrozole, letrozole or exemestane] were also resistant to NACT. These highly proliferating endocrine resistant tumors treated with conventional CT had a pCR rate of 5%, a pCR rate of > 20% was the predefined efficacy threshold.

In agreement with this, results at Arteaga's lab(14) showed that resistant tumors in the ACOSOG Z1031B were enriched in an E2F4 signature. This signature is prognostic of poor outcomes and can be downregulated in patients by 2-week treatment with PAL (POP trial(15, 16). Interestingly, in BC cell lines resistant to estradiol deprivation, CDK4/6i but not paclitaxel or fulvestrant, were able to completely suppress the expression of all genes composing the E2F4 signature.

In the neoMONARCH study(17) (NCT02441946), abemaciclib, alone or in combination with anastrozole as NA in EBC, reduced Ki67 expression after 2 weeks of treatment based on geometric mean change and patients with complete cell cycle arrest (Ki67 < 2.7%)(18, 19). A total of 224 postmenopausal women with stage I–IIIB HR-positive/HER2-negative BC were enrolled. The primary objective evaluated change in Ki67 from baseline to 2 weeks of treatment. Of the 224 ITT patients, baseline tumor Ki67 expression was available for 208 (93%) with Ki67 ≥ 5% in 195 (87%) of the tumors. Ki67 expression was assessed at baseline and after 2 weeks of treatment in tumors from 75% of patients including 80% in the combination arm, 69% in the abemaciclib arm, and 76% in the anastrozole arm. Seventy-eight patients achieved a Ki67 < 2.7%: 67.8% patients treated with abemaciclib + anastrozole, 57.7% patients treated with abemaciclib and 14.3% patients treated with anastrozole (P < 0.001). The ORR (overall response rate) by radiologic assessment at the completion of the study treatment (16 weeks) was of 46.4% and the pCR rate was of 3.7%. These data support continued evaluation of abemaciclib in EBC patients. Abemaciclib will probably succeed in getting FDA/ European Medicines Agency (EMA) indication for EBC ER-positive/HER2 negative disease through the MONARCH-E trial in adjuvant setting. However, the indication of abemaciclib will be very focused on "aggressive tumors" and mostly after CT as standard of care (SoC).

Recent results from TAILOR-Rx(20) and MINDACT(21) trials show that CT can be spared in patients with low or intermedium risk of recurrence. Patients with High-RS (Recurrence Score) (> 30) (Oncotype Dx®) benefit from CT, in part because those patients harbor tumors that are resistant to standard ET. The margin benefit of CT in high-RS ER-positive BC could be lower if compares to a more efficacious ET regimen (ET/abemaciclib). Neoadjuvant setting provides an ideal scenario to test this hypothesis and reach a go/no-go signal for an adjuvant study. Once new biological therapies in combination with ET are

available, an adequate patient selection for the different biological therapies is warranted.

Study Treatment:

Eligible patients will be randomized 1:1 to receive one of the following treatments:

Control Arm (Arm A):

- Doxorubicin 60mg/m^2 and cyclophosphamide 600mg/m^2 (AC) every 21 days for 4 cycles followed by
- weekly paclitaxel 80mg/m^2 for 12 weeks **or** 3-weekly docetaxel 100mg/m^2 for 4 cycles.

Approximately duration of 24 weeks (6 months).

In Arm A, luteinizing hormone-releasing hormone (LHRH) analogs are not allowed in premenopausal women.

Experimental Arm (Arm B):

- Letrozole 2.5mg orally daily + abemaciclib 150mg orally every 12 hours on a continuous dosing schedule plus LHRH analogs in premenopausal women, up to 12 months (±14 days), with study visits each 28 (±3) days from first study dose.

After neoadjuvant treatment in each arm, patients will undergo breast surgery, with or without +/- regional lymph nodes surgery, of the primary breast cancer. Adjuvant CT will be allowed as per investigator's judgment based on the assessment of the pathological tumor response.

Those patients who do not complete the neoadjuvant treatment according to the study protocol because of progression of the disease (PD), unacceptable toxicity or informed consent withdrawal, will be discontinued from the study treatment and treated as per investigator's judgment and only survival follow-up and death dates (if applicable) will be collected.

Additionally, in those patients who experience recurrence during the follow-up period of the study, survival follow-up will also be collected.

In survival follow-up, only dates on which the patient is alive or not will be collected.

Study Design and Treatment:

Study Design

This is an international, multicenter, open-label, randomized phase II study in the neoadjuvant setting.

Approximately 200 pre- and postmenopausal women with HR-positive/HER2 negative BC

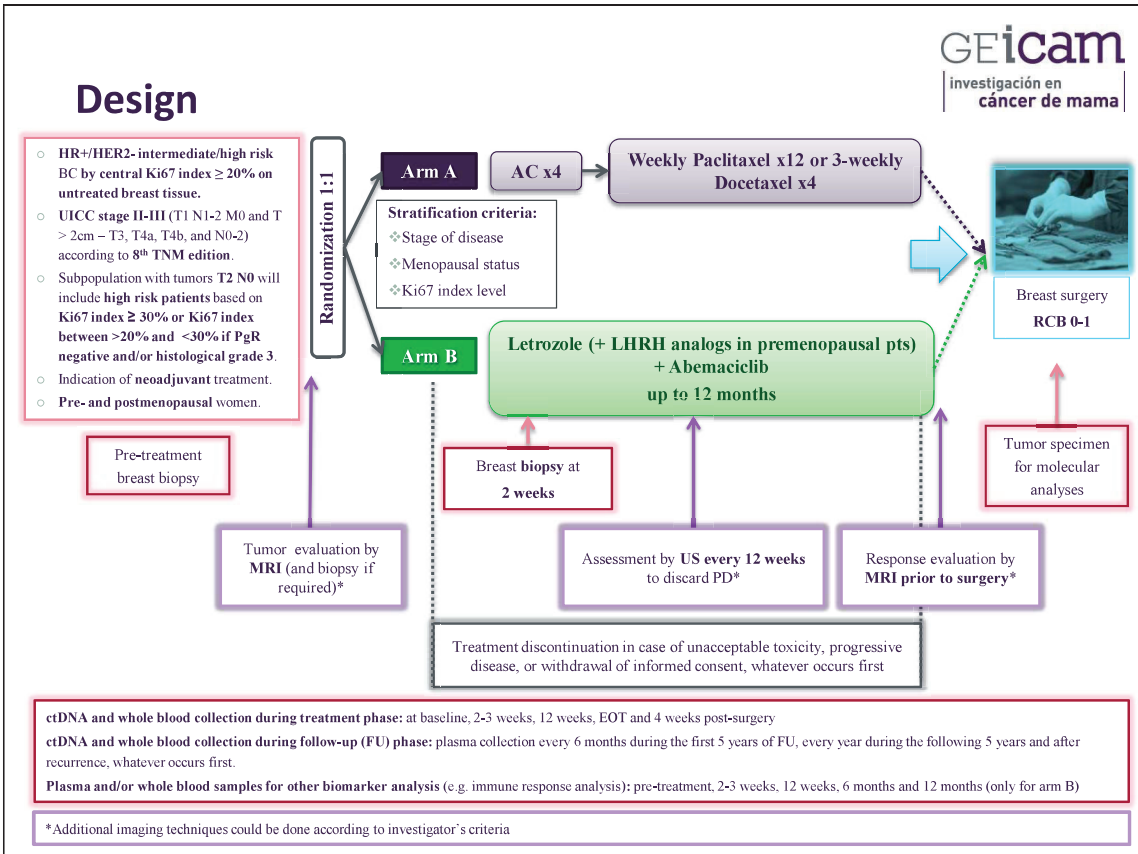
of intermediate/high risk determined by Ki67 index ≥ 20% on untreated breast tissue and centrally assessed, with indication of neoadjuvant treatment, will be included. Patients with EBC on stages II-III (T1c N1-2 M0 and tumor size (T) > 2cm – T3, T4a, T4b, and lymph node involvement (N) N0-2) according to the 8th edition of the UICC TNM Classification(22). The subgroup with tumors T2N0 will include high risk patients based on Ki67 index ≥ 30% or Ki67 index ≥ 20% and < 30% if progesterone receptor (PgR) negative and/or histological grade 3.

Patients will be stratified according to the disease stage (II vs. III), menopausal status (premenopausal vs. postmenopausal) and Ki67 index (Ki67 < 30% vs. Ki67 ≥ 30%).

Once the screening process (locally at site and at the central laboratory) is completed, fully eligible patients will be randomized in a 1:1 fashion to the control arm with standard CT based on anthracyclines and taxanes or to the experimental arm with letrozole + abemaciclib (+ LHRH analogs in premenopausal women).

All patients will be treated according to the stipulations below, unless any of the following occur: unacceptable toxicity, progressive disease, or withdrawal of informed consent, whatever occurs first.

After the last dose of any of the drugs in the neoadjuvant combinations***, in both treatment arms definitive surgery will be performed.*** For Arm A not earlier than 21 days and not later than 42 days after the last dose of chemotherapy, and for Arm B within 7 days from the last dose of abemaciclib and/or letrozole, unless toxicities are not recovered completely in any treatment arm.



Primary Objective:

- To assess the Residual Cancer Burden (RCB) 0-I rate in both treatment arms.

Primary End-point:

- Evaluation of the number of patients with a Residual Cancer Burden (RCB) 0-I index as a measure of efficacy. RCB(23) is a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden. It is estimated from routine pathological sections of the primary breast tumor site and the regional lymph nodes after the completion of NA therapy. The pathological variables include bidimensional diameters of the primary tumor bed, the proportion of primary tumor area containing invasive carcinoma, the number of positive lymph nodes, and the diameter of the largest nodal metastasis.

Secondary Objectives:

- Changes in Ki67 index value after 2 weeks of treatment in both treatment arms.
- RCB 0+I vs. RCB-II vs. RCB-III in both treatment arms (TNM downstaging).
- Changes in RCB value between both treatment arms.

- Rate of PEPI score 0 at surgery in both treatment arms.
- Clinical response measured by magnetic resonance imaging (MRI) according to RECIST v1.1 in both treatment arms.
- Rate of breast conserving surgery (BCS) in both treatment arms.
- iEFS (invasive Event Free Survival) in both treatment arms.
- Assessment of safety profile by National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0 classification.
- To assess molecular downstaging for high risk genomic groups defined by a multigene expression panel.

Secondary End-points:

- Percentage of decrease in the geometric mean of Ki67 index value after 2 weeks of treatment in both treatments arms.

  Number of patients with cell cycle arrest (Ki67 < 2.7%) after 2 weeks of treatment in both treatment arms.

- RCB is classified in four classes based on the residual disease (RD):
  - RCB-0 defined as pathological complete response.
  - RCB-I defined as minimal RD.
  - RCB-II defined as moderate RD.
  - RCB-III defined as extensive RD.
- Variation of RCB value based on the RD between both treatment arms.
- PEPI (Preoperative Endocrine Prognostic Index)(24) requires pathological stage (tumor size and nodal status), level of Ki67 protein and Allred ER score measured on the surgical specimen. PEPI score 0 includes pT1 or pT2, pN0, Ki67 ≤ 2.7%, Allred score > 2.
- Clinical Response Rate (CRR) is defined as the proportion of subjects with complete or partial radiographic response. Complete response (CR) and partial response (PR) definitions are assessed by MRI at baseline and prior to breast surgery, with or without regional lymph nodes surgery, and categorized according to percent reduction in tumor size.
- Rate of breast conserving surgery (BCS): defined as the proportion of patients who achieved BCS between both treatment arms.
- Invasive event free survival (iEFS): defined as time from randomization to progressive disease (PD) or invasive disease recurrence (local, regional, distant,

or contralateral), or death from any cause. Invasive disease recurrence is defined as:

- Ipsilateral invasive breast tumor recurrence (including second primary invasive breast cancer): an invasive breast cancer involving the same breast parenchyma as the original primary lesion.
- Ipsilateral regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, other regional lymph nodes, chest wall, and/or skin of the ipsilateral breast).
- Distant recurrence (i.e., evidence of breast cancer in any anatomic site outside local and/or regional location and that has been either histologically confirmed or clinically diagnosed as recurrent invasive breast cancer).
- Contralateral invasive breast cancer.
- Second primary invasive cancer of non-breast origin.

- Safety will be assessed by standard clinical and laboratory tests (hematology, serum chemistry). AEs grade will be defined by the NCI-CTCAE version 5.0. AEs terms will be coded according to MedDRA dictionary.
- Gene expression data provided by a multigene expression panel in sequential tumor biopsies.

Exploratory Objectives:

- Correlation of Ki67 protein level change after 2 weeks of treatment with some efficacy variables.
- Genetic changes in sequential tumor samples and tumor evolution during NA therapy, and its correlation with some efficacy variables and surrogate endpoints for response.
- To explore T-cell functional activation, immune suppression, neoantigens and cytokines production as well as other immune response biomarkers (such as CD4/CD8/FOXP3/PDL-1), in order to understand the activity of abemaciclib on the tumor microenvironment and the immune response.
- Circulating tumor DNA (ctDNA) dynamics as a surrogate endpoint for response and prognostic implications.
- Sequential genomic profiling and quantification of ctDNA samples to explore early response dynamics, minimal residual disease, tumor tracking, tumor mutational burden and clonal diversity along NA therapy and treatment follow up.
- To investigate if ctDNA dynamics after 2-3 weeks of treatment predicts PEPI score,

molecular downstaging, and minimal residual disease in ctDNA samples, and its correlation with some efficacy variables.

- To investigate if ctDNA dynamics after 3-4 weeks post-surgery predicts PEPI score and molecular downstaging, and its correlation with some efficacy variables.
- Genetic changes in pre vs. post NA therapy tumor samples to identify potential genomic mechanisms of resistance.
- To describe ctDNA mutation landscape in resistant tumors.
- Generation and expansion of *ex vivo* organoid-based models and/or xenographs from post-therapy tumors and if possible, from baseline tumors to perform high-throughput functional screening studies.
- Identification of indirect biomarkers of tumor biology and treatment activity by metabolomic analysis (glutamine pathway).
- To explore other possible biomarkers of clinical activity in tumor and blood samples.

Exploratory End-points:

- Variation of the Ki67 levels, determined by IHC, between pre-treatment and after 2 weeks-treatment biopsies, and its correlation with some efficacy variables.
- Mutational and copy number variation (CNV) data analyzed in pre-treatment and surgery tumor samples (large targeted gene panels including *TP53* and *MYC* mutations and *MYC* CNVs) and its correlation with some efficacy variables.
- Gene expression data provided by a multigene expression panel in sequential tumor biopsies and its correlation with some efficacy variables.
- Monitoring of ctDNA abundance and specific tumoral mutation and genetic changes observed in ctDNA samples along treatment and follow up.
- Mutations and CNV detected in ctDNA collected after recurrence.
- Values of other proteins, metabolites, RNA or DNA alterations obtained from the tissue or blood samples and its correlation with efficacy variables could be used for assessment of biomarkers related to activity of abemaciclib and breast tumor sensitivity and/or resistance to treatment.

Study population and main inclusion and exclusion criteria:

Pre- and postmenopausal women with HR positive/HER2 negative intermediate/high risk BC determined by central Ki67 index ≥ 20% on untreated breast tissue, with indication of

neoadjuvant treatment will be included.

Inclusion Criteria:

Patients are eligible to be enrolled in the study only if they **meet all** of the following criteria:

1. Written informed consent prior to any specific study procedures.
2. Women ≥ 18 years of age.
3. Documentation of histologically confirmed primary invasive adenocarcinoma of the breast. Adenocarcinoma with another component of epithelial origin (for example, medullary or neuroendocrine) is allowed.
4. Availability of a primary tumor tissue sample obtained during the diagnostic process before treatment for the central assessment of Ki67 index and biomarker exploratory analyses (following the specifications described in the Sample Management Manual of the study).
5. Documentation of HR positive and HER2 negative BC based on local laboratory determination.
    a. HR positive is defined as more than or equal to 10% positive cells by IHC for ER and/or progesterone receptor (PgR).
    b. HER2 negative tumor is determined according to recommendations of ASCO/CAP 2018 guidelines.
6. Intermediate and high risk patients based on Ki67 index value (≥ 20%) determined at a central laboratory.
7. Patients should be in the following clinical stages of disease according to the 8th edition of the TNM Classification of Breast Cancer by the UICC (Union for International Cancer Control): T1c N1-2 M0 and T2 (> 2cm) – T3, T4a, T4b, N0 – N2, M0 (stages IIA, IIB, IIIA or IIIB). Subpopulation with tumors T2 N0 M0 will include high risk patients based on Ki67 index ≥ 30% or Ki67 index between ≥ 20% and < 30% if PgR negative and/or histological grade 3.
8. Patients with diagnosis of suspicious for multifocal or multicentric breast cancer will be eligible for the study.. At least two tumor lesions should be biopsied. All histologically available tumor lesions must comply with the inclusion criterion no. 5.
    a. If all lesions have similar morphological characteristics (i.e. based on local assessment of type, grade, Ki67 index level, etc.…), only the largest tumor lesion will be assessed for central assessment of Ki67 index level.

b. If lesions have different morphological characteristics, discordant tumor lesions will be centrally evaluated for Ki67 index level. Patients will be eligible if at least one tumor lesion complies with criteria 6 and 7.

9. Indication of neoadjuvant treatment.
10. At the time of presentation, patients must be candidates for potentially curative surgery by surgeon's assessment.
11. Sentinel lymph node biopsy (SLNB) will be preferable after the neoadjuvant treatment. Those patients with SLNB before the neoadjuvant treatment will be eligible for the study only if the SLNB has a negative result (N0). One Step Nucleic Acid Amplification (OSNA) method is not allowed.
12. Pre- and postmenopausal women.

    Postmenopausal status is defined as:

    - Patient underwent bilateral oophorectomy, or
    - Age ≥ 60 years, or
    - Age < 60 years and amenorrhea for 12 or more months (in the absence of chemotherapy, tamoxifen, toremifene or ovarian suppression) and Follicle-stimulating hormone (FSH) and plasma estradiol are in the postmenopausal ranges per local normal ranges.

    All women who do not meet the criteria for postmenopausal status are considered premenopausal for the purpose of this trial.
13. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1.
14. Patients are able to swallow oral medications.
15. Adequate organ and bone marrow function:

    - ANC ≥ 1,500/mm^3 ($1.5x10^9$/L);
    - Platelets ≥ 100,000/mm^3 ($100x10^9$/L);
    - Hemoglobin (Hgb) ≥ 8g/dL (80g/L) (erythrocyte transfusions are permitted; initial treatment must not begin earlier than the day after the erythrocyte transfusion);
    - Total serum bilirubin ≤ 1.5xULN (≤ 2xULN and direct bilirubin within normal limits if Gilbert´s disease);
    - AST and ALT ≤ 3xULN.
16. Left ventricular ejection fraction (LVEF) ≥ 50% measured by multiple-gated

acquisition scan (MUGA) or echocardiogram (ECHO).

17. For premenopausal women: agreement to remain abstinent or use single or combined non-hormonal contraceptive methods that result in a failure rate of < 1% per year during the treatment period and for at least 3 weeks after the last dose of study treatment. Abstinence is only acceptable if it is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.

    Examples of non-hormonal contraceptive methods with a failure rate of < 1% per year include tubal ligation, male sterilization, and certain intrauterine devices. Alternatively, two methods (e.g., two barrier methods such as a condom and a cervical cap) may be combined to achieve a failure rate of < 1% per year. Barrier methods must always be supplemented with the use of a spermicide.

18. Negative serum pregnancy test within 7 days of the first dose of abemaciclib or chemotherapy for premenopausal women, and for women who have experienced menopause onset < 12 months prior to first dose of therapy.
19. Patients consent to biological sample provision for biomarker exploratory analyses.
20. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

Exclusion Criteria:

Patients will be excluded from the study if they **meet any** of the following criteria:

1. Previous anti-cancer treatment with therapeutic intent for current breast cancer is not allowed.
2. Patients with inflammatory breast cancer or synchronous bilateral invasive breast cancers are not eligible.
3. Serious and/or uncontrolled preexisting medical condition(s) that, in the judgment of the investigator, would preclude participation in this study (for example, interstitial lung disease, severe dyspnea at rest or requiring oxygen therapy, severe renal impairment [e.g. estimated creatinine clearance < 30ml/min], history of major surgical resection involving the stomach or small bowel, or preexisting Crohn's disease or ulcerative colitis or a preexisting chronic condition resulting in baseline Grade 2 or higher diarrhea).
4. Patients with rare hereditary problems of galactose intolerance, total lactase deficiency or glucose- galactose malabsorption.

5. Females who are pregnant or lactating.
6. Active systemic bacterial infection (requiring intravenous [IV] antibiotics at time of initiating study treatment), fungal infection, or detectable viral infection (such as known human immunodeficiency virus positivity or with known active hepatitis B or C [for example, hepatitis B surface antigen positive]. Screening is not required for enrollment.
7. Personal history of any of the following conditions: syncope of cardiovascular etiology, ventricular arrhythmia of pathological origin (including, but not limited to, ventricular tachycardia and ventricular fibrillation), or sudden cardiac arrest.
8. Diagnosis of any other malignancy within 5 years prior to randomization, except for adequately treated basal cell or squamous cell skin cancer, or carcinoma in situ of the cervix, breast or colorectal.
9. Prior hematopoietic stem cell or bone marrow transplantation.
10. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

Justification of Sample size determination and interim analysis:

A Bayesian design has been used to define the most appropriate sample size. The major advantage of this Bayesian approach is to allow us to evaluate how similar response rates between both treatment arms are, without using a very large non-inferiority study.

Comparability of RCB0/1 will be declared if the following Bayesian criterion is achieved in the primary RCB0/1 analysis:

$$\text{Posterior P}(\text{true RCB0/1}_{\text{Chemo}} - \text{true RCB0/1}_{\text{Abema+AI}} < 5\%) > 80\%$$

Full detail regarding the Bayesian model for ORR (including prior specifications) will be provided in the statistical analysis plan (SAP).

Simulations were conducted to evaluate probability of declaring comparability based on various scenarios of underlying true RCB0/1 values. Different underlying true RCB0/1 values for abemaciclib plus an AI were considered in the scenarios.

Three scenarios were explored (n=150, n=200, and n=250) and it is considered that the 2nd scenario with a sample size of 200 patients is the best option. Simulation results are shown in the table below:

$RCB0/1_{Chemo}$	$RCB0/1_{Abema+AI}$	N=150	N=200	N=250
0.16	0.16	57%	60%	61%
0.16	0.20	77%	82%	86%
0.16	0.24	90%	94%	96%
0.16	0.28	96%	98%	99%

Statistical analyses:

- Demographics and baseline characteristics:
  - Standard descriptive statistics, such as the mean, median, range and proportion, will be used to summarize the patient sample and to estimate parameters of interest. Ninety-five percent confidence intervals will be provided for estimates of interest wherever possible.
- Safety analyses:
  - Adverse events data and serious adverse events will be reported in frequency tables (overall and by intensity). The safety analysis will be performed in the safety population. Serious AE and deaths will be provided in a listing. All AEs resulting in discontinuation, dose modification, dosing interruption, and/or treatment delay of medicinal product will also be listed.
- Efficacy analyses:

  The final efficacy analyses will be performed in the ITT. The primary endpoint is the Residual Cancer Burden (RCB) 0-I rate in both treatment arms. Comparability between the two treatment arms will be assessed using the following Bayesian criterion:

  $$\text{Posterior } P(\text{true } RCB0/1_{Chemo} - \text{true } RCB0/1_{Abema+AI} < 5\%) > 80\%$$

  Posterior medians and Bayesian credible intervals will be reported for the RCB0/1 rate for each arm. In addition, arms will be compared using a Cochran-Mantel-Haenszel (CMH) test using the stratification factors. The accompanying 2-sided 95% confidence intervals (CIs) in each arm and the difference between arms will be computed.
- Biomarker analyses:
  - Biomarker exploratory analyses would include at least gene expression analysis to determine intrinsic subtypes and gene signatures of interest in sequential tumor samples, characterization of mutational genetic profiles and monitoring residual disease and tumor burden in tumor and ctDNA samples

	along the study, as well as proteomics and metabolomics analyses. All biomarkers will be correlated with efficacy endpoints, as feasible.
Study Duration: The start date of study is the date of the first site activation. Recruitment period will occur during approximately 2 years from first patient in (FPI). There would be a follow-up period of 10 years. The end date of study is the date of the last visit of the last patient (LPLV) including follow-up. Performing exploratory analyses will be independent of the date of the end of the study.	

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AE	Adverse Event
AEMPS	Spanish Agency for Medicines and Health Products
AESI	Adverse Event of Special Interest
AI	Aromatase Inhibitor
ALT/ALAT (SGPT)	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
AR	Adverse Reaction
AST/ASAT (SGOT)	Aspartate Aminotransferase
AUC	Area Under the Curve
BC	Breast Cancer
BCS	Breast Conservative Surgery
BID	Twice A Day
CBP	Child Bearing Potential: premenopausal women, and for women who have experienced menopause onset < 12 month prior to first dose of therapy.
cCR	Clinical Complete Response
CDK	Cyclin-Dependent Kinase
ctDNA	Circulating tumoral DNA
CI	Confidence Interval
Compliance	Adherence to all the trial-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
CR	Complete Response
CT	Chemotherapy
CTCAE	Common Terminology Criteria for Adverse Events
CT Scan	Computed Tomography Scan
DDI	Drug-Drug Interaction

DFS	Disease-Free Survival
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSUR	Development Safety Update Report
EBC	Early Breast Cancer
eCRF	Electronic Case Report Form (sometimes referred to as Clinical Report Form). An electronic form for recording study participants' data during a clinical study, as required by the protocol.
ECG	Electrocardiogram
ECHO	Echocardiography
ECOG	Eastern Cooperative Oncology Group
End of Study (Trial)	The end of study (trial) is the date of the last visit or last scheduled procedure shown in the Study Schedule for the last active patient in the study, including follow-up
Enroll	The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned a registration number and treatment.
ER	Estrogen Receptor
ER1	Estrogen Receptor 1
ER-LBD	Estrogen Receptor-Ligand Binding Domain
EC/IRB	Ethics Committee/Institutional review board: A board or committee (institutional, regional, or national) composed of medical professional and nonmedical members whose responsibility is to verify that the safety, welfare, and human rights of the patients participating in a clinical trial are protected.
ESR	Expedited Safety Report
ET	Endocrine Thearapy
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin-Embedded
GCP	Good Clinical Practice
GEICAM	Spanish Breast Cancer Group

G-CSF	Granulocyte Colony-Stimulating Factor
GI	GastroIntestinal
HER2	Human Epidermal Growth Factor Receptor 2
Hgb	Hemoglobin
HR	Hormone Receptor or Hazard Ratio depending on the context
IA	Interim Analysis
ICD	Informed Consent Document
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
Investigator	A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
ISH	In Situ Hybridization
ITT	Intent To Treat
Legal Representative	An individual, judicial, or other body authorized under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical trial.
LLN	Lower Limit of Normal
LFT	Liver Function test
MBC	Metastatic Breast Cancer
MID	Minimally important difference
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MUGA	Multigated Acquisition Scan
NA	Neoadjuvant
NCI	National Cancer Institute
NACT	Neoadjuvant Chemotherapy

NAET	Neoadjuvant Endocrine Therapy
NSAI	Non-Steroidal Aromatase Inhibitor
OR	Objective Response
ORR	Objective Response Rate
OS	Overall Survival
Patient	A subject with a defined disease
pCR	Pathological Complete Response
PD	Progressive Disease or Pharmacodynamic depending on the context
PEPI	Preoperative Endocrine Prognostic Index
PFS	Progression-Free Survival
PgR	Progesterone Receptor
PK	Pharmacokinetic
PR	Partial Response
PS	Perfomance Status
QD	Once A Day
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RECIST	Response Evaluation Criteria in Solid Tumors
RCB	Residual Cancer Burden
RR	Response Rate
RS	Recurrence Score
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAI	Steroidal aromatase inhibitor
SAP	Statistical Analysis Plan
SC	Steering Committee

Screen	The act of obtaining informed consent for participation in a clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients screened into a trial are those who sign the informed consent document directly or through their legally acceptable representatives.
SD	Stable Disease
SGOT	Serum Glutamate-Oxoloacetate Transaminase
SGPT	Serum Glutamate-Pyruvate Transaminase
SLN	Sentinel Lymph Node
SMM	Sample Management Manual
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TN	Triple Negative
TNM	Tumor, Node, Metastasis, for the purpose of staging
TTP	Time To Progression
ULN	Upper Limit of Normal
US	Ultrasound
VTE	Venous Thromboembolism
WNL	Within Normal Limits

Protocol GEICAM/2019-01

Phase II, randomized, open-label, international, multicenter study to compare efficacy of standard chemotherapy vs. letrozole plus abemaciclib as neoadjuvant therapy in HR-positive/HER2-negative high/intermediate risk breast cancer patients

"CARABELA Study"

1. Introduction

1.1. Overview of Breast Cancer

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among women, with an estimation of 2 million new cases and more than 600,000 deaths in 2018, worldwide(25). Breast cancer is a heterogeneous disease with multiple clinical, histopathological and genomics characteristics(26, 27). The hormone receptor-positive (HR-positive), human epidermal growth factor receptor 2-negative (HER2-negative) is the more common subtype and accounts for approximately 70% of all breast cancers(28). Gene expression profiling studies have classified breast tumors into several distinct biological subtypes with prognostic and therapeutic implications. According to this classification, four main molecular subtypes have been identified: Luminal A, Luminal B, HER2-enriched, and Basal. The molecular subtypes of breast cancer correspond reasonably well to a classification based on immunohistochemistry (IHC) markers on the basis of HR status , HER2 status and proliferation markers (ie, Ki67)(29). The most common subtypes are HR-positive (ER-positive and/or PgR-positive)/HER2-negative, comprising Luminal A-like and Luminal B-like subtypes. Either a high Ki67 (≥ 20%) or a low PgR (< 20%) value may be used to distinguish between Luminal A-like (more endocrine sensitive, indolent, better prognosis) and Luminal B-like (less endocrine sensitive, more aggressive, worse prognosis) tumors(30, 31). Luminal A–like tumors are ER-positive and HER2-negative with low Ki67 expression (< 14%) or with intermediate Ki67 expression (14% to 19%) and high PgR levels (> 20%). Luminal B–like tumors are ER-positive and HER2-negative with intermediate Ki67 expression (14% to 19%) and low PgR levels (< 20%) or with high Ki67 expression (≥ 20%)(32). The vast majority of patients with luminal breast cancer are diagnosed at an early stage of their disease which are potentially curable with locoregional treatments such as surgery and radiation(33). After surgery, HR-positive early breast cancer (EBC) is usually treated with adjuvant endocrine therapy (ET) and, depending on the estimated individual risk of disease recurrence, adjuvant chemotherapy (CT)(34). Most validated clinical and pathological features that may indicate a higher risk of disease recurrence, and therefore the need for adjuvant CT, include large primary tumor size and involvement of axillary lymph nodes. Other prognostic factors are, histopathological grade, PgR expression, Ki67 expression, multi-gene testing recurrence scores, age and comorbidities. Using these factors, EBC can be classified as having low, intermediate/moderate or high risk for recurrence after surgery(35). Patients with smaller tumors, no axillary involvement and Luminal A-like characteristics or low recurrence genomic score, derive little benefit, if any, from the addition of adjuvant CT to ET. On the other hand, patients with metastases in multiple axillary lymph nodes, Luminal B-like tumors or high recurrence genomic score have a higher risk of recurrence and are usually considered for adjuvant CT, in addition to ET(20,

21). With current standard of care adjuvant therapy, approximately 25-30% of women with high risk early stage HR-positive breast cancer experience recurrence(36, 37). Consequently, there is a critical need for more optimal adjuvant therapy in patients with early HR-positive breast cancer who have a high likelihood of distant recurrence.

1.2. Neoadjuvant Treatment for HR-positive Breast Cancer

Neoadjuvant treatment of breast cancer is increasingly used to improve the chance of breast conservation and as a platform for the development of investigational drugs, triaging of novel combinations, biomarker validation, and discovery of mechanisms of drug resistance. In contrast to adjuvant setting, neoadjuvant CT (NACT) remains the first treatment option for most HR-positive/HER2-negative breast cancer patients and neoadjuvant endocrine therapy (NAET) is commonly relegated for elderly patients who are unsuitable for CT. The efficacy of NACT depends on the regimens used. Anthracyclines and taxanes are the most active group of CT regimens used for BC patients(38). Currently only 3% of eligible patients in the United States receive NAET treatment(37). However, two randomized phase II trials of NAET vs. NACT showed a similar response and rate of breast conservation for both treatment arms, with substantially less toxicity with ET. Although 3 to 4 months has been the standard duration of most trials of NAET, there's seem to be a consensus that this length of treatment is insufficient to reach maximal tumor response. Non-randomized studies suggest that some tumors benefit from a longer duration (6–12 months) of anti-ER treatment(39-41).

Recently, an increasing number of NAET are testing new drugs in combination with ET in patients with HR-positive BC(2). Conducting these trials are more challenging than trials of NACT and/or anti-HER2 therapy in patients with triple negative (TN) or HER2-positive BC, respectively. A main limitation is that pathological complete response (pCR), a well-stablished biomarker in TN and HER2-positive BC(3), is uncommon after 3 to 4 months of NAET and not an effective surrogate of long-term outcome in patients with HR-positive/HER2-negative BC(42, 43). Therapy-induced changes in the proliferation marker Ki67 and the preoperative endocrine prognostic index (PEPI), a composite score of post-treatment ER, Ki67, tumor size, and axillary nodal status, are widely used markers of response to antiestrogens in NAET trials. A high post-treatment Ki67 score and a high PEPI score have been shown to correlate with an increased risk of recurrence(13, 24, 44, 45). The Residual Cancer Burden (RCB) index is another biomarker of response to NACT that is increasingly used in NAET studies. The RCB index evaluates 5 post-treatment variables: two-dimensional tumor bed, cellularity, percentage of carcinoma in situ (CIS), number of metastatic lymph nodes, and the diameter of the largest nodal metastases. It classifies the surgical specimen into four categories: RCB-0 (pCR), RCB-I (minimal residual disease), RCB-II (moderate residual disease), and RCB-III (extensive residual disease). RCB is able to predict risk of recurrence after NACT, which is highest for RCB-

III (53.6%) and similar for RCB-0 and RCB-I (2.4% and 5.4%, respectively)(23, 46). Interestingly, the incorporation of Ki67 into RCB improved the prognostic ability of either Ki67 and RCB alone(47).

The preoperative therapy setting is an excellent clinical research platform where treatment can be compared and triaged using endpoints that correlate with long-term outcome. There are multiples examples where NAET trials results predicts outcome from adjuvant and metastatic studies(2). An illustrative example supporting the value of the NA platform for clinical trial prioritization is provided by IMPACT trial(45) comparing anastrozole, tamoxifen, or the combination for 12 weeks. In IMPACT, following 2 and 12 weeks of treatment, anastrozole suppressed Ki67 by 76% and 82%, respectively, compared with tamoxifen by 59% and 62%, and the combination of both drugs by 64% and 61%. These differences paralleled the outcome of the same three treatment arms in the large adjuvant ATAC trial which enrolled more than 9,000 women(48). After a median follow-up of 30 months, anastrozole significantly improved disease-free survival (DFS) over tamoxifen and the combination, whereas DFS was similar in the tamoxifen and combination arms. It can be argued that had the results of IMPACT been known before the ATAC trial, these data would have provided a rationale for elimination of the combination arm in ATAC trial, thus significantly reducing the size, duration, and cost of the adjuvant study.

1.3. Role of Abemaciclib in the Treatment of Breast Cancer

1.3.1 Abemaciclib in Advanced Breast Cancer

The treatment of metastatic HR-positive/HER2-negative BC has changed since the approval of cyclin-dependent kinases (CDK) 4 and 6 inhibitors (CDK4/6i). These drugs, in combination with ET, have consistently and homogeneously demonstrated doubling the progression free survival (PFS) interval (from approximately 14 to 28 months in the first line), increase of response rates and delay in the start of CT. All these benefits are achieved with minimal toxicity and an excellent quality of life perceived by patients(20, 21, 36, 37, 39, 40).

Abemaciclib is a potent and selective small-molecule CDK4/6i administrated orally on a twice daily continuous schedule. Abemaciclib is structurally distinct from other CDK4/6i (such as ribociclib and palbociclib) and is 14 times more potent against cyclin D1/CDK4 than against cyclin D3/CDK6 in enzymatic assays(49). Currently, abemaciclib is FDA-EMA approved for the treatment of advanced HR-positive/HER2-negative BC in three scenarios:

1) As first line treatment, based on MONARCH-3 trial(50): a randomized (2:1), double-blinded, placebo-controlled, multicenter clinical trial in postmenopausal women with

HR-positive/HER2-negative BC. A total of 493 patients were randomized to receive either abemaciclib 150mg or placebo orally twice daily, plus physician's choice of letrozole or anastrozole. The estimated median PFS was 28.2 months (95% CI: 23.5, not reached) for patients receiving abemaciclib and 14.8 months (95% CI: 11.2, 19.2) for those receiving placebo (HR 0.540; 95% CI: 0.418, 0.698; $p < 0.0001$).

2) As second line treatment, based on MONARCH-2 trial(51) : a randomized, placebo-controlled, multicenter trial in women with HR-positive/HER2-negative metastatic breast cancer (MBC), disease progression following ET and who had not received CT in the metastatic setting. A total of 669 patients received either abemaciclib or placebo orally twice daily plus fulvestrant 500mg on days 1 and 15 of cycle 1 and then on day 1 of cycle 2 and beyond (28-day cycles). Median PFS for patients taking fulvestrant with abemaciclib was 16.4 months compared with 9.3 months for those taking fulvestrant with placebo (HR 0.553; 95% CI: 0.449, 0.681; $p < 0.0001$). The objective response rate (ORR) in patients with measurable disease taking fulvestrant with abemaciclib was 48.1% (95% CI: 42.6, 53.6) compared to 21.3% (95% CI 15.1, 27.6) in the fulvestrant with placebo treated patients.
3) As third or greater line, based on MONARCH-1 trial(52): a single-arm, open-label, multicenter study in women with measurable HR-positive/HER2-negative MBC whose disease progressed during or after ET, had received a taxane in any setting, and one or two prior CT regimens in the metastatic setting. A total of 132 patients received abemaciclib 200mg orally twice daily on a continuous schedule until PD or unmanageable toxicity. Objective response rate was 19.7 percent (95% CI: 13.3, 27.5) with a median response duration of 8.6 months (95% CI: 5.8, 10.2). This indication is only included in the FDA approval.

Abemaciclib is indicated for the treatment of HR-positive, HER2-negative advanced or metastatic BC:

- in combination with an AI,
- in combination with fulvestrant, and
- as a single agent following progressive disease (PD) after ET and CT regimens.

Refer to the local label for information regarding the precise indications approved in a specific country or region.

The recommended dose of abemaciclib in combination with ET is 150 mg orally, BID on a continuous dosing schedule. The recommended dose of abemaciclib as a single agent is 200 mg orally, BID on a continuous dosing schedule. It is recommended that treatment be continued until PD or unacceptable toxicity. Abemaciclib may be taken with or without food. Abemaciclib is available as 50-, 100-, 150-, and 200-mg tablets.

Management of some adverse reactions may require dose interruption and/or dose reduction. If dose reduction is necessary, decrease the dose by 50 mg at a time. Discontinue abemaciclib for patients unable to tolerate 50 mg BID.

Three registration studies (MONARCH 3, MONARCH 2, and MONARCH 1) provide the primary safety data supporting abemaciclib use in combination with endocrine therapies and as a single agent. Adverse events of special interests (AESIs) were determined to be events that were:

- of clinical significance
- potentially associated with other agents that inhibit CDK4 and CDK6, or
- observed in preclinical evaluation or earlier clinical studies of abemaciclib, although in some instances a causal association with abemaciclib could not be clearly established.

The following AEs are considered to be AESIs for abemaciclib:

- neutropenia,
- infections,
- diarrhea,
- hepatic events, including increases in AST and ALT,
- venous thromboembolic events (VTEs), and
- ILD/pneumonitis.

Overall, the safety profile of the three registration studies was generally consistent in terms of adverse event (AE) incidence and severity. The most frequent toxicities were of low grade (grade 1 or 2), generally predictable, manageable, and reversible. The most commonly reported treatment-emergent adverse events (TEAE) included diarrhea, neutropenia, fatigue, nausea, vomiting, abdominal pain, decreased appetite, and anemia. The most common AEs leading to dose reductions, dose omissions, or discontinuation of abemaciclib included diarrhea and neutropenia. Overall, the safety profile of abemaciclib remains unchanged based on the completed and ongoing studies.

- *Diarrhea* was the most commonly treatment-emergent adverse event (TEAE) with abemaciclib in the reported studies. Incidence was greatest during the first month of abemaciclib treatment and was lower subsequently. The median time to onset of the first diarrhea event was approximately 6 to 8 days across studies, and the median duration of diarrhea was 9 to 12 days (Grade 2) and 6 to 8 days (Grade 3) across abemaciclib studies in combination with ET. Exposure-response analyses demonstrated that dose was the most important factor related to diarrhea. There was 1 Grade 5 (fatal) event of diarrhea reported in the EBC Study JPCF which occurred in the setting of acute intra-abdominal sepsis. Diarrhea returned to baseline or lesser grade with supportive treatment such as loperamide and/or dose adjustment. At the first sign of

loose stools, treatment with antidiarrheal agents, such as loperamide, should be initiated.

- *Neutropenia* has been observed in studies with single-agent abemaciclib and when combined with ET and was predominantly of low-grade severity. It was reported frequently (45.1%) and a Grade 3 or 4 decrease in neutrophil counts (based on laboratory findings) was reported in 28.2% of patients receiving abemaciclib in combination with aromatase inhibitors (AIs) or fulvestrant. The median time to onset of Grade 3 or 4 neutropenia was 29 to 33 days, and median time to resolution was 11 to 15 days. On central laboratory analysis, neutrophil count decrease from the baseline visit was observed; mean neutrophil counts generally remained stable at the later visits and were reversible once patients discontinued the treatment. Febrile neutropenia was reported in 0.9% patients. Dose modification is recommended for patients who develop Grade 3 or 4 neutropenia.
- *Increased aminotransferases* in patients receiving abemaciclib in combination with AIs or fulvestrant, ALT and AST elevations were reported frequently (15.1% and 14.2%, respectively). Elevations in ALT/AST were predominantly of Grade 1 or Grade 2 severity, and a concurrent increase in blood bilirubin was infrequent. Generally, ALT and AST increases were manageable by dose reduction or dose omission and resolved upon discontinuation of study treatment. Several patients had isolated episodes of elevated ALT and AST that resolved without dose adjustment. Grade 3 or 4 ALT or AST elevations (based on laboratory findings) were reported in 6.1% and 4.2% patients. The median time to onset of Grade 3 or 4 ALT elevation was 57 to 61 days, and median time to resolution was 14 days. The median time to onset of Grade 3 or 4 AST elevation was 71 to 185 days, and median time to resolution was 13 to 15 days. Dose modification is recommended for patients who develop ALT or AST increase of Grade 3 or 4 or experience persistent or recurrent Grade 2.
- *Venous thromboembolic events (VTE):* in randomized Phase 3 studies in patients with ABC or EBC treated with abemaciclib in combination with ET, a greater number of patients experienced VTE events in the abemaciclib plus ET arm than in the placebo plus ET arme or ET alone arm. Patients were treated with low molecular weight heparin (LMWH) and generally, these events did not result in discontinuation of the study treatment. 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 ETendocrine therapy is known to be associated with the occurrence of VTEs.
- *Interstitial lung disease (ILD)/Pneumonitis:* in randomized Phase 3 studies, in patients with ABC, EBC, and in patients with stage IV non-small cell lung cancer, ILD/pneumonitis events, including ILD, pneumonitis, organizing pneumonia, and pulmonary fibrosis, were reported. The majority of events were Grade 1 or Grade 2,

with serious cases and fatal events reported in each study. Most of the events were confounded due to factors such as lung metastases, prior exposure to radiation, presence of infection, and/or administration of other medication (e.g., prior chemotherapeutic agents known to cause pneumonitis). Patients who develop new or worsening pulmonary symptoms such as dyspnea, cough, and fever should be investigated and treated as per local clinical practice and/or guidelines. Investigations may include imaging, such as high resolution computed tomography (HRCT), broncheoalveolar lavage (BAL), and biopsy as clinically indicated. Discontinue abemaciclib in cases of severe (Grade 3 or 4) ILD/pneumonitis. In cases of non-severe ILD/pneumonitis, the benefit of resuming abemaciclib treatment must be carefully evaluated.

- *Ocular effects:* in rodents, cataracts/lens fiber degeneration and retinal atrophy have been observed at clinically relevant abemaciclib exposure levels. Patients who experience changes in vision should undergo ophthalmological investigations as clinically indicated.
- *Creatinine*: although not an AR, abemaciclib has been shown to increase serum creatinine in 98.3% of patients (based on laboratory findings), 1.9% Grade 3 or 4 (based on laboratory findings). In patients receiving an AI or fulvestrant alone, 78.4% reported an increase in serum creatinine (all laboratory grades).
- *Carcinogenicity*: in a 2-year carcinogenicity study in rats, interstitial (Leydig) cell hyperplasia and benign interstitial (Leydig) cell adenomas in the testes were observed at clinically relevant exposure levels. Male patients should be instructed to conduct regular self-examination of their testicles and report any new symptoms or changes.
- *Reproductive safety*: abemaciclib has not been studied in pregnant or lactating women; therefore, no clinical data are available. Animal studies indicate that abemaciclib has the potential to cause fetal harm when administered to a pregnant woman. Male animals given abemaciclib had injury to their testes; therefore, male patients should be advised about the possibility of infertility.

Table 1. Tabulated List of Adverse Reactions

In the following table, adverse reactions (ARs) are listed in order of MedDRA body system organ class and frequency. Frequency gradings are: very common (≥ 1/10), common (≥ 1/100 to < 1/10), uncommon (≥ 1/1,000 to < 1/100), rare (≥ 1/10,000 to < 1/1,000), very rare (< 1/10,000), and not known (cannot be estimated from the available data). Within each frequency grouping, ARs are presented in order of decreasing seriousness.

System organ class *Frequency* Preferred term	Abemaciclib plus ET[a]		
	All Grades Toxicity (%)	**Grade 3 Toxicity (%)**	**Grade 4 Toxicity (%)**
Infections and infestations			
Very common			
Infections[b]	43.6	5.2	1.0
Blood and lymphatic system disorders			
Very common			
Neutropenia	45.1	22.9	2.5
Leukopenia	25.7	8.5	0.3
Anemia	30.1	7.0	0.1
Thrombocytopenia	14.3	2.2	1.0
Common			
Lymphopenia	7.3	3.0	0.1
Uncommon			
Febrile neutropenia	0.9	0.7	0.1
Metabolism and nutrition disorders			
Very common			
Decreased appetite	26.4	1.3	0
Nervous system disorders			
Very common			
Dysgeusia	14.3	0	0
Dizziness	12.9	0.5	0
Eye disorders			
Common			
Lacrimation increased	6.8	0.1	0
Vascular disorders			
Common			
Venous thromboembolism[c]	5.3	1.7	0.3
Gastrointestinal disorders			
Very common			
Diarrhea	84.6	11.7	0
Vomiting	27.7	1.2	0
Nausea	43.5	2.1	0
Skin and subcutaneous tissue disorders			
Very common			
Alopecia Pruritus	20.7	0	0
Rash	13.5	0	0
Common	12.9	1.0	0
Dry skin	9.0	0	0
Musculoskeletal and connective tissue disorders			
Common			
Muscular weakness	8.3	0.5	0

System organ class *Frequency* Preferred term	Abemaciclib plus ET[a]		
	All Grades Toxicity (%)	**Grade 3 Toxicity (%)**	**Grade 4 Toxicity (%)**
General disorders and administration site Conditions			
Very common			
Fatigue	40.5	2.3	0
Pyrexia	10.7	0.1	0
Investigations			
Very common			
Alanine aminotransferase increased	15.1	4.8	0.3
Aspartate aminotransferase increased	14.2	2.9	0

a. Abemaciclib in combination with letrozole, anastrozole, or fulvestrant.

b. Infections include all preferred terms (PTs) that are part of the System Organ Class Infections and infestations.

c. Venous thromboembolic events include deep venous thrombosis (DVT), pulmonary embolism, cerebral venous sinus thrombosis, subclavian, axillary vein thrombosis, DVT inferior vena cava and pelvic venous thrombosis.

1.3.2 Abemaciclib in Early Stage Breast Cancer

The exciting results observed with CDK4/6i in the treatment of advanced HR-positive/HER2-negative BC patients have activated the evaluation of these agents in the early-stage setting. Several studies have explored the efficacy of CDK4/6i and ET over ET alone as preoperative treatment. In all of them, the combination induced a more potent cell cycle arrest than ET alone(6, 19, 53, 54).

NeoMONARCH (NCT02441946) was a multicenter, randomized, open-label, phase 2 study comparing the biological effects of abemaciclib in combination with anastrozole to those of abemaciclib monotherapy and anastrozole monotherapy for women with early-stage HR-positive/HER2-negative BC. The study met its primary endpoint demonstrating that abemaciclib alone and in combination with anastrozole significantly reduced Ki67 expression after 2 weeks of treatment compared to anastrozole alone. Complete cell cycle arrest (Ki67 < 2.7%) after two weeks of treatment was 68% for abemaciclib and anastrozole and 14% for anastrozole monotherapy. After 16 weeks of treatment with the combination, 53% of patients achieved a radiological response (partial or complete response) and 7 patients (3.7%) obtained a pCR. Subgroup analyses demonstrated that abemaciclib efficacy was not associated with disease stage, baseline lymph node involvement, tumor grade, tumor size, or *PIK3CA* mutation status. The high activity of abemaciclib in the NA setting supports a large ongoing phase III adjuvant trial (monarchE) that evaluates the potential for abemaciclib to enhance adjuvant ET. MonarchE is a

multicenter, randomized, open-label, phase III study assessing the efficacy of twice-daily intake of 150mg abemaciclib for 2 years plus adjuvant ET (5-10 years) vs. adjuvant ET alone (5-10 years), in terms of invasive disease-free survival (iDFS).

MonarchE (NCT03155997) is an ongoing study including high risk early stage BC defined as: pathological tumor involvement in ≥ 4 axillary lymph nodes, or in 1 to 3 and high histologic/nuclear grade 3 or have pathological primary tumor size ≥ 5 cm or Ki67 index of ≥ 20%. Overall, based on results from the second interim analysis (IA), the safety profile of abemaciclib plus ET in this Phase 3 study was acceptable, monitorable, manageable, and generally consistent with that previously reported for abemaciclib and ET. Diarrhea and neutropenia were 2 of most common Grade ≥ 3 TEAEs in abemaciclib plus ET treated patients, with diminishing frequencies over time. The overall incidence of all grade TEAEs, Grade ≥ 3, and SAEs in men was similar to that of the overall population.The efficacy results described here are based on the second efficacy interim analysis using a data cut-off date of 16 March 2020. The primary objective of monarchE was invasive disease-free survival (iDFS). At the time of the second efficacy IA, monarchE met the primary objective of the study, with abemaciclib plus ET achieving statistically significant and clinically meaningful improvement in iDFS compared to ET alone in the overall ITT population (hazard ratio [HR] 0.747, 95% confidence interval [CI] 0.598, 0.932, p=0.0096). There was also a clinically meaningful improvement in 2-year iDFS rates for patients treated with abemaciclib plus ET. At the time of the second efficacy IA, iDFS in patients with high Ki-67 was tested sequentially after iDFS in ITT population being statistically significant, with a pre-specified 2-sided alpha level of 0.02. Statistical significance was not met for iDFS in the high Ki-67 population and will be tested again at the time of the final iDFS analysis.

Distant relapse-free survival (DRFS): at the time of the second efficacy IA, abemaciclib plus ET demonstrated a numerical benefit in DRFS compared to ET alone, with a clinically meaningful 2-year DRFS rate improvement in the ITT population. The DRFS results indicated the treatment benefit in terms of delaying metastatic disease by 28.3% compared to ET alone and were consistent with the statistically significant iDFS results in the ITT population.

Overall survival: at the time of the second efficacy IA, OS was immature, with large variability on the OS HR estimate. Overall survival will be re-evaluated at further analysis time points.

1.4. Overview of Abemaciclib

1.4.1 Preclinical Data

The cell cycle is the process by which mammalian cells replicate their deoxyribonucleic acid (DNA) and undergo cellular division. The mammalian cell cycle has 4 phases: S phase, in which DNA replication occurs; M (mitosis) phase, in which the replicated DNA and cellular components are divided to form 2 daughter cells; G2 phase (after S phase), in which the cell prepares for mitosis; and G1 phase (after mitosis), in which cells commit to another round of DNA and cellular replication. Defects in the pathways that regulate cell proliferation in response to mitogenic signaling and other extracellular stimuli such as cell density and nutrients are a hallmark of cancer cells(55) and the G1 restriction point (R) is believed to be essential to maintain control of cell proliferation(56, 57). A primary mechanism controlling cellcycle progression through the restriction point is the CDK4 and CDK6 pathway (CDK4 and CDK6-cyclin D-INK4-Rb) and the importance of this pathway in regulating cell proliferation is highlighted by inactivation of restriction point control in a majority (> 85%) of human tumors(58) . CDK4 and CDK6 regulate the G1 restriction point through phosphorylation of the Rb protein, which allows subsequent expression of genes required to complete transition through G1 and initiate DNA replication. Selective inhibition of CDK4 and CDK6 results in a reversible arrest of cancer cells at the restriction point when used as a single agent in cells containing functional Rb protein. Abemaciclib mesylate is a potent inhibitor of CDK4 and CDK6 that is selective over other CDKs at the enzyme and cellular level. This is demonstrated in Colo-205 cells by potent cellular inhibition of Rb phosphorylation (pSer780, 50% inhibition concentration [IC50] = 120 ± 36 nM) and by exclusive G1 cell cycle arrest (indicated by accumulation of cells with 2N DNA content) up to 6 µM concentration. Other studies have confirmed and demonstrated that abemaciclib mesylate inhibits CDK4 and CDK6 to induce G1 arrest specifically in Rb-proficient tumors. Using *in vitro* kinase panel screening, abemaciclib mesylate also demonstrates inhibition (IC50 < 0.3 µM) of the human protein kinases hCDK9, hPIM1, hPIM2, hHIPK2, hDYRK2,GSK3ß, hCDK5/P35, and CK2; however, the reversible G1 arrest seen *in vitro* and *in vivo* indicates that the inhibition of CDK4 and CDK6 by abemaciclib mesylate predominates over these other activities. Notably, an analysis of the human circulating metabolites showed that 3 of them had activity against the target that was nearly equivalent to the parent compound. In particular, metabolites M2 and M20 were evaluated *in vitro* in biochemical enzyme assays and cell-based functional assays. In biochemical assays,both metabolites showed nearly identical potency (IC50 between 1 and 3 nM) as that of abemaciclib. Likewise, both metabolites inhibited cell growth and cell cycle progression in a concentration-dependent manner that was consistent with the inhibition of CDK4 and CDK6. In the cancer cell lines evaluated, metabolite LSN3106726 consistently showed potencies nearly identical to

abemaciclib while LSN2839567 was approximately 2- to 3-fold less potent. Like abemaciclib, both LSN2839567 and LSN3106726 also induced senescence in addition to growth inhibition in breast cancer cell lines at similar concentrations. Metabolite LSN3106729 (M18) is a minor metabolite and also active, but its potency in functional assays was 3- to 20-fold lower than abemaciclib. The evaluation of abemaciclib in animal models shows that the phenotypic selectivity for G1 arrest, which is observed in cell culture studies, is also observed in the *in vivo* xenograft tumors. For such studies, direct biochemical inhibition of CDK4 and CDK6 as well as the phenotypic inhibition of cell cycle progression can be assessed in tumor explants by measuring Rb phosphorylation at serine 780 (pRb), since Rb is a direct target of CDK4 and CDK6 activity. Reduced pRb expression is also a phenotypic marker for G1 arrest at the restriction point (R). The inhibition of TopoIIa and phospho-histone H3 (pHH3) provides additional measures for the inhibition of cell cycle progression through the S and M phases, respectively. The inhibition of CDK4 and CDK6 and cell cycle progression *in vivo* by abemaciclib mesylate is evident by the dose-dependent reduction of pRb in the Colo-205 xenografts. These effects on Rb also correlate with similar dose-dependent reductions in the other cell markers TopoIIa and pHH3, indicating that inhibition of CDK4 and CDK6 activity by abemaciclib mesylate results in the inhibition of cell cycle progression in the Colo-205 xenograft tumors. This inhibition of pRb and the cell cycle also shows a time-dependency, whereby abemaciclib mesylate treatment results in a sustained pharmacodynamic response in a mouse Colo-205 xenograft model. Specifically, a 50mg/kg oral dose resulted in =50% inhibition of Rb phosphorylation for 1 to 24 hours after dosing. This effect also correlated with an inhibition of cell cycle progression as indicated by the potent suppression of pRb, TopoIIa, and pHH3 observed at 24 hours following dosing. A dose response for inhibition was observed in these studies such that the threshold effective doses for 70% inhibition for pRb and TopoIIa inhibition 24 hours after oral dosing were 14.1 and 14.3mg/kg, respectively. Abemaciclib mesylate demonstrates significant inhibition of tumor growth in multiple murine xenograft models of human cancer including Colo-205 (colorectal cancer), NCI-H460 (non n.small-cell lung cancer [NSCLC]), U87 MG (glioblastoma), and JeKo-1 (mantle cell lymphoma [MCL]). Although characterized by a different constellation of genomic mutations, each of these 4 human xenografts has an intact, functional Rb tumor-suppressor protein. Xenograft growth inhibition was in general dose-dependent from 15 to 100mg/kg following daily oral administration for 21 days. Consistent with the mechanism of action of abemaciclib, the antitumor activity in Colo-205 was associated with a sustained inhibition of pRb, TopoIIa, and pHH3. As a result of its brain exposure, treatment with abemaciclib mesylate produces a statistically significant and dose-dependent improvement in survival when assessed in a rat orthotropic brain tumor model. Tumor cells were implanted intracerebrally and compound treatment started 4 days after implantation (20, 40, or 80mg/kg daily for 21 days). Efficacy was assessed using Kaplan-Meier survival analysis. Statistically significant improved survival was observed after treatment at 40 and 80mg/kg.

The results in the JeKo-1 MCL model demonstrate the potential value for targeting specific cancers with known alterations in the CDK4- and CDK6-cyclin D-INK4-Rb pathways. This model is typical of MCLs that overexpress cyclin D1 as a result of a specific chromosomal translocation [t(11;14)(q13;q32)]. The overexpression of cyclin D1, which leads to activation of CDK4 and CDK6, is an important driver for the disease and correlates with the sensitivity of this xenograft model to inhibition of CDK4 and CDK6 by abemaciclib mesylate. In particular, complete growth inhibition was observed at a dose as low as 15mg/kg, with a decrease in baseline tumor burden (ie, regressions) occurring when the dose was increased to 25 and 50mg/kg. The sustainability of the antitumor response was also dose-dependent in that the delay for tumor regrowth following the cessation of dosing increased as the dose of abemaciclib mesylate was increased. In particular, no tumor regrowth was observed during the entire 3-week period following cessation of treatment at 50mg/kg. Studies in breast cancer cell lines have elucidated potential tailoring strategies based on histological and genetic characteristics associated with human breast cancer. These particular studies, which evaluated *in vitro* growth inhibition across a diverse panel of 46 breast cell lines representing the known molecular subgroups of breast cancer, indicated that sensitivity to abemaciclib was greater in estrogen receptor-positive (ER-positive) lines with luminal histology. In accordance with the known biology for CDK4 and CDK6, the results also indicated that many of these sensitive cell lines are also characterized as having amplification of CCND1, which is the gene that encodes cyclin D1. Moreover, the cell lines that showed the least sensitivity to abemaciclib were those that had either homozygous deletions of RB1, the gene which encodes Rb, or copy number amplifications in E2F3. Both of these alterations presumably mitigate the effects of CDK4 and CDK6 inhibition, since both Rb and E2F3 are regulatory nodes for the G1 to S transition that are downstream from CDK4 and CDK6. These cell culture observations were further validated by studies *in vivo* in mice bearing human breast cancer xenografts such as ZR-75-1, which is a xenograft model for ER-positive and CCND1-amplified breast cancer. Specifically, the treatment of mice bearing ZR-75-1 xenograft tumors with either 50 or 75mg/kg resulted in either a complete inhibition of tumor growth or reductions in tumor size (regression). Notably, these effects on tumor growth correlated with the dose-dependent inhibition of CDK4 and CDK6 and cell cycle progression, as indicated by the inhibition of pRb and cell cycle biomarkers such as TopoIIα or pHH3. Non-clinical studies also show that abemaciclib can be used effectively in combination with standard cytotoxic or targeted therapies to improve the efficacy of these agents. In these studies, the combination therapies, compared to the single-agent treatments, resulted in either a greater inhibition of tumor growth during therapy or in a longer duration of growth inhibition following the cessation of treatment. Additionally, studies in ER-positive human breast cancer xenograft models show that abemaciclib can combine effectively with endocrine treatments such as tamoxifen or fulvestrant to attain greater inhibition of tumor growth when compared to monotherapy with any of these agents.

To support human clinical studies, the toxicity profile of abemaciclib has been effectively characterized in mice, rats, and dogs through a package of repeat-dose toxicology, safety pharmacology, developmental and reproductive toxicology, carcinogenicity, phototoxicity, and genetic toxicology studies. These studies demonstrate an acceptable safety profile with toxicities that are generally considered to be monitorable and reversible. The safety profile of abemaciclib in non-clinical toxicology studies is generally consistent with the adverse effects (AEs) observed to date in humans.

Primary target organs:

- The primary target organs associated with daily dosing of abemaciclib observed in at least 2 species (rats and dogs) are bone marrow, pancytopenia and hypocellularity
- gastrointestinal tract – crypt necrosis/hyperplasia and villous atrophy
- eye – cataracts and retinopathy
- lymphoid tissues – lymphoid depletion, and
- male reproductive tract – hypospermatogenesis and atrophy in the testes.

With the exception of eye effects, these effects were consistent with antiproliferative effects in rapidly dividing cells.

Eye effects have only been observed in rodents. After 3 months of treatment in mice, eye effects were observed only in female mice at a dose level that exceeded the maximum tolerated dose (MTD). In rats, the eye effects were observed in studies which were 6 months in duration or longer. The effects were generally more severe in male rats, but do appear in female rats as well.

Other general toxicity findings:

In rats, the lung was also identified as a target organ for toxicity, characterized by multifocal macrophage accumulation with or without reversible bronchoalveolar inflammation. Only in the 6 month rat study, minimal inflammation, observed concurrently with vacuolated macrophages, within heart valves was observed in male rats treated at 30 mg/kg/day.

Reproductive effects:

In pregnant rats exposed to abemaciclib, skeletal and cardiac variations and malformations were observed along with decreased fetal weights; thus, abemaciclib is teratogenic in rats.

Despite the lack of effects on fertility in male rats, abemaciclib may impair fertility in men based on testicular injury observed in mice, rats, and dogs treated with abemaciclib. Abemaciclib had no effects on fertility in female rats nor any effects on female reproductive organs.

Carcinogenic effects:

Benign interstitial (Leydig) cell tumors were observed in the testes of rats treated for up to 95 weeks with abemaciclib. These findings were accompanied by interstitial cell hyperplasia; however, interstitial cell hyperplasia has not been observed in rats treated for 6 months or less.

1.4.2 Human Pharmacokinetic (PK) Data

The PK profile of abemaciclib was studied using data available from a total of 222 patients enrolled in study I3Y-MC-JPBA (JPBA). In the dose range of 50 to 275 mg, abemaciclib absorption is slow, with a Tmax ranging from 4 to 6 hours and AUC and Cmax generally increased in a dose-proportional manner after a single dose and also with multiple twice-daily doses. With repeated administration of 200 mg twice daily, which was identified as the maximum tolerated dose (MTD) in study JPBA, abemaciclib accumulated with a geometric mean accumulation ratio of 2.5 (67% coefficient of variation [CV]). At steady state (SS), after repeated oral Q12H dosing of 200 mg, the mean minimum plasma concentration at steady state (Cmin,ss) and maximum observed plasma concentration at steady state (Cmax,ss) were 197 and 298 ng/mL, respectively. The mean area under the steady state plasma concentration–time curve over a dosing interval (AUC0-τ,ss) was approximately 3000 ng·hours/mL. The mean absolute bioavailability (study I3Y-MC-JPBS [JPBS]) of abemaciclib after a single oral dose of 200 mg is 45%. In a study (I3Y-MC-JPBU [JPBU]) of the effect of a high-fat meal using the 25% w/w capsule formulation, abemaciclib exposure increased by 27% (90% confidence interval [CI] 1.18, 1.36) based on AUC, and by 35% (90% CI 1.25, 1.46) based on Cmax. A similar result was observed in study JPCC, in which the effect of food on the PK of the tablet formulation was evaluated. Abemaciclib exposure increased by 13% (90% CI 1.05, 1.22) based on AUC, and by 30% (90% CI 1.20, 1.40) based on Cmax. Although the change in AUC and Cmax in both studies was statistically significant, it was small relative to the variability in exposure in the cancer patient population, and therefore not considered to be clinically relevant. In both studies, a high-fat meal did not change the interindividual variability in PK. Therefore, *abemaciclib can be taken without regard to meals*. New protocols no longer contain requirements regarding food restrictions.

Summary

After single and multiple BID twice-daily 50- to 275 mg doses of abemaciclib,

- abemaciclib absorption was slow
- Tmax ranged from 4 to 6 hours
- t1/2 ranged from 17 to 38 hours, and
- area under the concentration versus time curve (AUC) and Cmax generally increased in a dose-proportional manner.

At 200 mg twice daily (BID), the maximum tolerated dose (MTD) for abemaciclib monotherapy when dosed BID, abemaciclib achieved

- a mean minimum plasma concentration at steady state (Cmin,ss) of 197 ng/mL

- a mean maximum observed plasma concentration at steady state (Cmax,ss) of 298 ng/mL, and
- a mean area under the steady-state plasma concentration–time curve over a dosing interval (AUC0-τ,ss) of approximately 3000 ng·hours/mL.

The mean absolute bioavailability of abemaciclib after a single oral dose of 200 mg is 45%.

The relative bioavailability of the 25% w/w capsule formulation used in clinical development was compared to the commercially available tablet formulation. In 85 subjects, the PK after a single dose of 3 × 50-mg tablets was bioequivalent to the PK of a single dose of 3 × 50-mg capsules, with

- an AUC0-∞ ratio of 1.02 (90% confidence interval [CI]: 0.986, 1.06) and
- a Cmax ratio of 1.03 (90% CI: 0.986, 1.08).

In a study of the effect of a high-fat meal using the 25% w/w capsule formulation, abemaciclib

- AUC increased by 27% (90% CI: 1.18, 1.36) and
- Cmax increased by 35% (90% CI: 1.25, 1.46).

Similarly, for the tablet formulation (Study JPCC), a high-fat meal

- increased AUC by 13% (90% CI: 1.05, 1.22) and
- increased Cmax by 30% (90% CI: 1.20, 1.40).

Although these changes were statistically significant, they were small relative to exposure variability in the cancer patient population and therefore not considered to be clinically relevant. In both studies, a high-fat meal did not change the interindividual variability in PK. Therefore, abemaciclib can be taken with or without food. New protocols no longer specifycontain requirements regarding food restrictions.

Distribution

In humans, Abemaciclib was highly bound to plasma proteins, serum albumin and alpha-1-acid glycoprotein with a mean bound fraction was approximately 96% to 98%, which was independent of concentration from 152 to 5066 ng/mL. The active metabolites were approximately 89% to 94% bound to plasma proteins. The geometric mean apparent volume of distribution at steady state (Vss/F) for abemaciclib was approximately 1300 L (96% CV).

Distribution to the Central Nervous System

Distribution of abemaciclib to the central nervous system in patients with brain metastases has been clinically evaluated by collection of

- cerebrospinal fluid (CSF) samples in Studies I3Y-MC-JPBA (JPBA) (N=10) and I3Y-MC-JPBO (Part C N=6; Part F N=3), and
- brain tumor tissues in Study JPBO (Part C N=8).

Abemaciclib demonstrated penetration of the blood brain barrier in cancer patients with detectable concentrations in brain tumor tissues and CSF.

These brain tumor tissue and CSF concentrations were

- consistently above the IC50 for CDK4 and CDK6, and
- comparable with plasma concentrations which are associated with clinical efficacy in the MONARCH metastatic breast cancer (mBC) studies.

Metabolism

In vitro and *in vivo* studies indicated that metabolism was the main route of elimination for abemaciclib. It is metabolized to several oxidative metabolites primarily by cytochrome P450 (CYP) 3A, with formation of LSN2839567 (M2) representing the major elimination pathway. Following oral administration of a single 150mg dose of [14C]abemaciclib to healthy subjects, abemaciclib was the major drug-derived entity in plasma (34%), with the metabolites LSN3106726 (M20), LSN2839567 (M2), and LSN3106729 (M18) accounting for approximately 26%, 13%, and 5%, respectively. Abemaciclib was extensively metabolized, with 7% of the dose recovered in the feces as unchanged drug. *In vitro*, the major metabolites LSN3106726 and LSN2839567 were active with similar potency as abemaciclib. The active metabolites were eliminated via biliary excretion directly and/or further metabolized and eliminated via biliary excretion. The elimination t1/2 of LSN2839567 and LSN3106726 in cancer patients at a dose of 200mg was 36 and 32 hours, respectively. Across a dose range of 50 to 200 mg, the elimination t1/2 and metabolite-to-parent drug ratio remained stable with dose for LSN2839567 and LSN3106726, suggesting that *metabolism of abemaciclib does not change with dose*.

Elimination

The geometric mean apparent oral clearance (CL/F) of abemaciclib was 38.3 L/hour (105% CV), and the mean (range) plasma elimination t1/2 for abemaciclib in patients at a dose of 200mg was 21 (12-63) hours. The mean t1/2 ranged from 17 to 38 hours, with no consistent trends related to dose, suggesting no dose-dependent change in CL. After a single oral dose of [14C]abemaciclib in 6 healthy subjects, approximately 81% of the dose was excreted in the feces and 3.4% excreted in urine. *The majority (52%) of the dose excreted in feces constituted metabolites, indicating that abemaciclib is highly metabolized*. Only 7% was recovered in the feces as unchanged drug.

CYP3A Inducers and Inhibitors

Please, refer to Section 5.6.1. of this document.

Abemaciclib is predominantly cleared by oxidative metabolism via CYP3A. Co-administration of abemaciclib with rifampin, a strong CYP3A inducer, decreased the potency-adjusted unbound AUC of abemaciclib plus its active metabolites by 77%.and may

lead to reduced activity. The percentage reduction in the potency-adjusted unbound AUC of abemaciclib plus its active metabolites in the presence of moderate CYP3A4 inducers modafinil, efavirenz, and bosentan was predicted to be 29%, 53%, and 41%, respectively, using physiologically based PK modeling.

As a result, concomitant use of CYP3A inducers may reduce the activity of abemaciclib.

At a minimum, it is recommended to avoid concomitant use of CYP3A inducers and consider alternative agents.

Co-administration of a strong CYP3A inhibitor (clarithromycin) resulted in a 2.5-fold increase in the potency-adjusted unbound AUC of abemaciclib plus its active metabolites in patients with advanced and/or metastatic cancer. The potency-adjusted unbound AUC of abemaciclib plus its active metabolites is predicted to increase by 7.1-fold, 3.8-fold, and 2.4-fold in the presence of CYP3A inhibitors ketoconazole, itraconazole, and diltiazem, respectively, using physiologically based PK modeling.

As a result, concomitant use of strong and moderate CYP3A inhibitors may increase the likelihood of AEs related to abemaciclib.

At a minimum, it is recommended to avoid concomitant use of strong CYP3A inhibitors.

Combinations with Endocrine Therapy and Anti-Cancer Agents

Abemaciclib has been studied in combination with fulvestrant, anastrozole, letrozole, tamoxifen, exemestane, everolimus, trastuzumab, pemetrexed, gemcitabine, LY3023414, galunisertib and ramucirumab(59, 60). No clinically significant PK drug interaction was observed with these agents. Overall, the safety results in the Phase 2 study (I3Y-MC-JPBO) was consistent with the known safety profile of abemaciclib with no new safety signals identified. Most TEAEs were of mild or moderate severity. Diarrhoea, fatigue, and nausea were the most common AEs for abemaciclib.

Hepatic Impairment

Abemaciclib is metabolized in the liver. The effect of hepatic impairment as defined by Child-Pugh on abemaciclib PK was evaluated in the Phase 1 study I3Y-MC-JPBV (JPBV). In subjects with severe hepatic impairment, the abemaciclib t1/2 increased from 24 to 55 hours. No dosage adjustement is required for patients with mild or moderate hepatic impairment, but changing the dosing frequency to once daily may be required for patients with severe hepatic impairment.

Renal impairment

The influence of baseline creatinine clearance on abemaciclib disposition was evaluated with 233 patients with normal renal function (Cockcroft–Gault creatinine clearance [CGCL] > 90 mL/min), 186 patients with mild renal impairment (60 mL/min ≤ CGCL < 90 mL/min), 64 patients with moderate renal impairment (30 mL/min ≤ CGCL < 60 mL/min), and none with severe renal impairment (15 mL/min ≤ CGCL < 30 mL/min; 0%). Baseline

creatinine clearance was not a significant covariate on abemaciclib disposition. No dose adjustment is necessary for patients with mild or moderate renal impairment. There are no data in patients with severe renal impairment or in patients on dialysis to provide any dose adjustment recommendation.

1.4.3 Pharmacodynamic and Biomarker Analyses

Pharmacodynamic assessments for CDK4 and CDK6 inhibition and cell cycle arrest downstream of the G1 restriction point were performed on skin biopsies collected from 180 patients enrolled in Study JPBA. The skin biopsies were collected immediately before dose and 4 hours after dose on Day 15 of Cycle 1 (i.e., at steady state after repeated every 24 hours and/or every 12 hours (Q12H) oral dosing of abemaciclib). The CDK4 and CDK6 inhibition and cell cycle arrest downstream of the G1 restriction point were explored by measuring the level of expression of pRb and TopoIIα, respectively.

Skin biopsies collected both before dose and 4 hours after dose show a decrease in the level of pRb expression with increasing plasma concentration. More specifically, more robust and more consistent CDK4 and CDK6 inhibition seems to be associated with abemaciclib plasma concentration levels exceeding 200 ng/mL. These findings were confirmed when pRb levels were expressed as a percentage of the baseline value measured before treatment initiation.

Likewise, a decrease in cell density in the S phase, as measured by a decrease in the level of TopoIIα expression, was observed with increasing plasma concentration. TopoIIα expression fell below its baseline value measured pretreatment for abemaciclib steady-state plasma concentration levels exceeding 200 ng/mL.

Altogether, these findings suggest that abemaciclib-mediated CDK4 and CDK6 inhibition and subsequent cell cycle arrest upstream of the G1 restriction point are time dependent and are associated with a minimum abemaciclib $C_{min,ss}$ of approximately 200 ng/mL.

It is notable that these findings are consistent with previous PK/pharmacodynamic analyses performed in Colo-205 xenograft tumors, wherein constant CDK4 and CDK6 inhibition and constant cell cycle arrest were associated with a similar abemaciclib plasma concentration level of approximately 200 ng/mL.

In Studies MONARCH 2 and MONARCH 3, a dynamic population PK (PopPK)/pharmacodynamic model incorporating individual dosing history, fluctuating concentrations, change in tumor size, and progression-free survival (PFS) was built to describe the relationship among dose, concentrations, and efficacy. There was a positive linear relationship identified between abemaciclib exposure and tumor shrinkage and also PFS. Larger tumor reductions and longer PFS were associated with higher abemaciclib concentrations within the concentration range observed in MONARCH 2 and MONARCH 3.

1.4.4 QTc Evaluation Data

Relationship between Plasma Concentrations of Abemaciclib and Metabolites and QT Interval

In Study JPCA, single ascending oral doses of 200, 300, 400, or 600 mg abemaciclib or placebo were administered to healthy subjects to determine the relationship between plasma concentrations of abemaciclib, its major active metabolites M2 and M20, and QT interval. Exposure-response analysis revealed that abemaciclib does not cause clinically significant Fridericia's corrected QT interval (QTcF) prolongation in healthy subjects as the upper bounds of the 2-sided 90% CI of the predicted baseline- and placebo-corrected change-from-baseline QTcF were below 10 ms at the highest observed abemaciclib, M2, M20, and total analyte concentrations.

1.4.5 Abemaciclib Dose Rationale

The following Phase 1 studies in advanced cancer and/or metastatic cancer (solid tumors) were conducted. Refer to Table 5.1 of the IB December 2018 for details of the below listed studies.

- I3Y-MC-JPBA: in the first part of this dose-escalation Phase 1 study (Part A), patients were treated with either 50, 100, 150, or 225 mg abemaciclib Q24H or 75, 100, 150, 200, and 275 mg abemaciclib Q12H to determine the MTD of abemaciclib. The MTD for the once-daily dose was not established. At abemaciclib 275 mg Q12H dose level, 2 of the 3 patients experienced dose-limiting toxicities (DLTs) of Grade 3 fatigue. Therefore, the identified MTD for the twice-daily dosing schedule was 200mg Q12H. This study demonstrated acceptable safety and tolerability for abemaciclib in patients with advanced cancers.
- I3Y-JE-JPBC: safety data from Study JPBC were similar in Japanese patients for single agent abemaciclib as those observed in Study JPBA. The MTD of 200mg Q12H was confirmed in Study JPBC.
- I3Y-MC-JPBE: this study assessed the effect of clarithromycin on the PK of abemaciclib and its metabolites. Single doses of 50mg abemaciclib administered during the drug-interaction phase of the study were generally well tolerated when administered alone or co-administered with 500mg clarithromycin Q12H and the TEAE profile was similar for each treatment.
- I3Y-MC-JPCP (abemaciclib monotherapy and impact of food on tolerability): overall, the safety and tolerability profile of abemaciclib 200 mg BID administered in various feeding states were acceptable in this patient population with no new safety signals identified. This study did not demonstrate any impact of food on the tolerability of abemaciclib in relation to the incidence of diarrhea and associated dose modifications. The incidence of severe diarrhea (Grade 3 and above severity)

observed in this study (4.2%) was lower compared to that reported in the MONARCH 1 (19.7%), MONARCH 2 (14.5%), and MONARCH 3 (9.5%) studies. Prolonged Grade 2 diarrhea was reported for 2 patients (8.3%) in Arm 1, 4 patients (17.4%) in Arm 2, and 5 patients (20.8%) in Arm 3.

1.5. Study Rationale

Currently chemotherapy (CT) is one of the standard neoadjuvant (NA) therapies in high risk luminal breast cancer (BC) patients. Nowadays there are several NA studies comparing CT vs. CDK4/6i + ET.

- NeoPalAna study(53) (NCT01723774): single-arm phase II trial to determine the antiproliferative activity of palbociclib when added to anastrozole in pre- and postmenopausal women with newly diagnosed clinical stage II/II ER-positive (Allred score 6-8), HER2-negative (0 or 1+ by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) negative) BC. The primary objective was to determine whether the addition of anastrozole to palbociclib induces a higher rate of complete cell cycle arrest (CCCA: Ki67 $\leq$ 2.7%) than that achieved by anastrozole alone as initial therapy at C1D15. The primary endpoint was chosen based on the long-term follow-up data of several neoadjuvant endocrine therapy (NAET) trials which demonstrated that the 2.7% Ki67 cut-point (natural log of one) during NAET is associated with favorable BC relapse free and overall survival (OS). The added effect of palbociclib over that of anastrozole was determined by analysis of tumor biopsies collected at C1D1 following 4 weeks of cycle 0 anastrozole monotherapy, and at C1D15, 2 weeks after the addition of palbociclib to anastrozole. CCCA and Ki67 responses were assessed by PIK3CA mutation status because of the alternative strategy of PIK3CA-targeted therapy in the mutation-positive population. Secondary objectives included analysis of CCCA and Ki67 response by baseline PAM50-based intrinsic subtypes, and assessment of clinical, radiological, and pathologic response and safety profiles. Exploratory biomarker studies included gene expression and somatic mutation profiling. The study was designed to ensure the sample size for the PIK3CA WT cohort and the overall population for the primary endpoint analysis. A sample size of 33 in the PIK3CA WT cohort was chosen based on the Fleming's single-stage phase II design to test the hypothesis that palbociclib plus anastrozole leads to at least 50% improvement over anastrozole alone in CCCA rates [44% with anastrozole based on historical data vs. 66% with palbociclib plus anastrozole, power 0.8, alpha 0.05]. The primary endpoint was met if more than 20 of 33 patients achieved CCCA.
  Between April 2013 and April 2015, 50 patients (18 pre- and 32 postmenopausal), median age 57.5 (range, 34.1–79.6) years, with clinical stage II/III ER-positive,

HER2-negative BC enrolled to the study, which included 16 in the PIK3CA Mut, 32 in the PIK3CA WT cohort, and 2 with unknown PIK3CA status. Thirty-nine patients completed NAT and underwent definitive breast and axillary surgery. Following the protocol amendment that added cycle 5 prior to surgery, 10 patients received cycle 5 palbociclib and all underwent surgery as scheduled. The rates of CCCA with palbociclib plus anastrozole were significantly higher at C1D15 than that at C1D1 with anastrozole monotherapy for all evaluable patients (87% vs. 26%, $P < 0.001$), PIK3CA Mut (100% vs. 25%, $P < 0.001$), and PIK3CA WT (79%vs. 25%, $P < 0.001$) cohorts. The CCCA rates at C1D15 exceeded the predefined cut-point for meeting the primary endpoint of at least 66% in the overall, PIK3CA WT, and Mut cohorts. Of the 31 patients resistant to anastrozole (non-CCCA at C1D1), 26 (84%) responded to palbociclib (CCCA at C1D15). When considered as a continuous variable, Ki67 levels were significantly reduced from baseline C0D1 to C1D1 following anastrozole monotherapy ($P < 0.01$) and from C1D1 to C1D15 after adding palbociclib ($P < 0.01$) for both PIK3CA WT and Mut cohorts. There was a greater variability in Ki67 response among the PIK3CA WT tumors. Palbociclib enhanced cell-cycle control over anastrozole monotherapy regardless of luminal subtype (A vs. B) and PIK3CA status with activity observed across a broad range of clinicopathologic and mutation profiles.

- N007 study(5) (NCT01709370): The purpose of the study was to test the efficacy of neoadjuvant palbociclib therapy and to evaluate its impact on cell cycle arrest and changes in EndoPredict (EP) scores before and after treatment. Postmenopausal women with histologically proven ER-positive, HER2−negative invasive BC, ≥ 2 cm, were enrolled in an open-label, single-arm study. Twenty eligible patients were given letrozole 2.5 mg per day together with palbociclib 125 mg per day for 3 out of 4 weeks in repeated cycles for 16 weeks (4 cycles) before surgery. Palbociclib was stopped 1 week before surgery, whereas letrozole was continued till day of surgery. The primary end points were clinical response rates (cRR) and preoperative endocrine prognostic index (PEPI). The secondary end points were pathologic response and gene expression testing with EP test on collected tumor samples.
  The following results were obtained: 17 patients showed a clinical response of ≥ 50%, including 8 complete responses (CRs) and 9 partial responses (PRs). There was significant reduction in area ($P < 0.0001$) and volume ($P = 0.017$) of the cancer. Pathologic complete response (pCR) was achieved in one patient; all cancers were downgraded after treatment. Ki67 ($P = 0.044$) and EP scores ($P < 0.0001$) were significantly reduced after treatment. The mean value of Ki67 before treatment was 21.7%; after treatment it was 11.4% ($P = 0.044$). Core biopsy was performed on 7 patients on day 15 of cycle 1 for the evaluation of Ki67. It was less than 2.7 in five of seven (71.4%) patients. However, only eight of 20 (40%) patients had a Ki67 less

than 2.7 at the time of surgery after treatment. Except for 3 patients with elevation after treatment, the other 17 patients showed a significant drop in value. Eight of 17 (47.1%) patients had a high proliferation fraction (Ki67 > 15%) before treatment but only three of 20 (15%) patients were determined to have Ki67 over 15% after treatment.

Analysis of the relative gene expression levels showed that all proliferative genes, IL6ST and RBBP8 were decreased after palbociclib treatment. 6 patients with intermediate and three patients with high PEPI risk scores were found to have low EPclin scores. All patients with high PEPI relapse risk score had high EPclin score. In conclusion, effective clinical response was demonstrated by neoadjuvant letrozole in combination with palbociclib. Compared with PEPI, EPclin might be a better parameter to estimate prognosis after neoadjuvant therapy.

- PALLET study(6) (NCT02296801): randomized phase II study to evaluate palbociclib in addition to letrozole as neoadjuvant therapy in ER-positive early BC. Postmenopausal women with tumors ≥ 2.0 cm were randomly assigned 3:2:2:2 to letrozole 2.5 mg/d for 14 weeks (A); letrozole for 2 weeks, then palbociclib plus letrozole for 14 weeks (B); palbociclib for 2 weeks, then palbociclib plus letrozole to 14 weeks (C); or palbociclib plus letrozole for 14 weeks (D). Palbociclib 125 mg/d was administered orally on a 21-days-on, 7-days-off schedule. Core-cut biopsies were taken at baseline and 2 and 14 weeks. Co-primary end points for letrozole vs. palbociclib plus letrozole groups (A v B + C + D) were change in Ki67 (protein encoded by the MKI67 gene; immunohistochemistry) between baseline and 14 weeks and clinical response (ordinal and ultrasound) after 14 weeks. Complete cell-cycle arrest was defined as Ki67 less than or equal to 2.7%. Apoptosis was characterized by cleaved poly (ADP-ribose) polymerase.

  Three hundred seven patients were recruited. Clinical response was not significantly different between palbociclib plus letrozole and letrozole groups (P = 0.20; CR + PR, 54.3% vs. 49.5%), and PD was 3.2% vs. 5.4%, respectively. Median log-fold change in Ki67 was greater with palbociclib plus letrozole compared with letrozole (-4.1 vs. -2.2; P < 0.001) in the 190 evaluable patients (61.9%), corresponding to a geometric mean change of -97.4% vs. -88.5%. More patients on palbociclib plus letrozole achieved CCCA (90% vs. 59%; P < 0.001). Median log-fold change (suppression) of cleaved poly (ADP-ribose) polymerase was greater with palbociclib plus letrozole vs. letrozole (-0.80 vs. -0.42; P < 0.001). More patients had Grade ≥ 3 toxicity on palbociclib plus letrozole (49.8% vs. 17.0%; P < 0.001) mainly because of asymptomatic neutropenia.
- NeoPAL study(7) (NCT02400567): randomized, parallel, non-comparative, proof-of-concept, phase II study. Patients with ER-positive, HER2 negative, Prosigna®-defined luminal B, or luminal A and nodal status (N) positive, stage II–IIIA BC, not

candidate for BCS (breast conserving surgery), were randomly assigned (1:1) to either letrozole (2.5 mg daily) and palbociclib (125 mg daily, 3 weeks on and 1 week off) during 19 weeks (4.8 months), or to CT (5-fluorouracil, epirubicin (100 mg/m^2) and cyclophosphamide (FEC100) with three 21-day courses followed by docetaxel 100 mg/m^2 with three 21-day courses). Breast surgery was carried out at day 1 of week 20. Sentinel lymph node (SLN) surgery was allowed only after completion of the NA therapy. Patients with pN0, pN0iþ, or pN1mi SLN were classified as pN0 for residual cancer burden (RCB) calculation. Patients who did not achieve RCB 0–I in letrozole/palbociclib arm were recommended to receive adjuvant CT. One hundred and six randomized patients had a median Prosigna® score of 71 (22-93), thus leading ~85% of tumors to be classified as 'high-risk'. The interim analysis mandated the accrual to be stopped early. The primary end point was RCB 0-1 rate on the ITT (intent to treat) population (locally assessed according to MDACC recommendations for which pathologists of participating centers were specifically trained): letrozole/palbociclib 7.7% (95% confident interval (CI) 0.4–14.9) and CT 15.7% (95% CI 5.7–25.7). This study did not reach the primary endpoint of 20% RCB 0-I after 19 weeks of treatment (7.7%; 95% CI 0.4-14.9). Other results of the study include: a) pCR: letrozole/palbociclib 3.8% and CT 5.9%; b) clinical response (75%) and BCS rates (69%) were similar in both arms; c) Ki67 index: baseline [median (range): 25% (1-80) on letrozole/palbociclib and 30% (2-80) on CT; geometric mean 24.1% vs. 27.7%] and final [median (range): 3% (1-40) on letrozole/palbociclib and 9% (2-15) on CT; geometric mean 1.17% vs. 3.7%]; d) PEPI (Preoperative Endocrine Prognostic Index) 0 score: letrozole/palbociclib 17.6% and 8% CT.

- CORALLEEN study(8) (NCT03248427): a parallel-arm, multicenter, randomized, open-label, phase II clinical trial for postmenopausal women with stage I-IIIA, HR-positive, HER2-negative, luminal B according to PAM50 intrinsic subtype, BC, with tumor size ≥ 2 cm by magnetic resonance imaging (MRI). Patients were randomly assigned (1:1) to receive NACT (adriamycin/doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 (AC) for 4 cycles followed by weekly paclitaxel 80 mg/m^2 for 12 weeks) vs. six 28-day cycles of letrozole 2.5 mg/day plus ribociclib 600 mg once daily for 3 weeks on, 1 week off, for 24 weeks. Primary endpoint was to evaluate the proportion of patients with PAM50 low risk of relapse (ROR) disease at surgery in the modified intention-to-treat (ITT) population including all randomly assigned patients who received study drug and had a baseline and at least one post-baseline measurement of ROR score. The PAM50 ROR risk class integrated gene expression data, tumor size, and nodal status to define prognosis.
  Between July 2017 to December 2018, 106 patients were enrolled. At baseline, of the 106 patients, 92 (87%) had high ROR disease (44 [85%] of 52 in the letrozole

plus ribociclib group and 48 [89%] of 54 in the CT group) and 14 (13%) patients had intermediate-ROR disease (eight [15%] and six [11%]). Median follow up was 200·0 days (IQR 191·2–206·0). At surgery, 23 (46·9%; 95% CI 32·5–61·7) of 49 patients in the letrozole plus ribociclib group and 24 (46·1%; 32·9–61·5) of 52 patients in the CT group were low-ROR. The most common Grade 3–4 adverse events (AEs) in the letrozole plus ribociclib group were neutropenia (22 [43%] of 51 patients) and elevated alanine aminotransferase (ALT) concentrations (ten [20%]). The most common Grade 3–4 AEs in the CT group were neutropenia (31 [60%] of 52 patients) and febrile neutropenia (seven [13%]). No deaths were observed during the study in either group.

Chemotherapy seemed to be superior to CDK4/6i in terms of objective response rate (ORR) assessed by MRI. However, more patients in the letrozole plus ribociclib arm received breast conserving surgery (BCS) compared to the CT arm. No differences between the two arms were suggested when ORR was evaluated by physical examination. Pathologic complete response (pCR) and residual cancer burden (RCB) of 0–I were low, with patients treated with CT appearing more likely to achieve pCR and RCB 0–I compared to the ones treated with the letrozole plus ribociclib arm.

Moving to biological response, the trial showed greater reduction in Ki67 expression levels with the cyclins-dependent kinase (CDK)4/6 inhibitor than with CT. Of note, this decrease in Ki67 could be underestimated due to Ki67 rebound occurring when the CDK4/6 inhibitor are suspended some days before surgery, as the NeoPalAna study previously showed. This issue is applicable to the CORALLEEN trial where 13.1 (standard deviation [SD] 14.6) was the mean number of days between the last dose of ribociclib and surgery.

The conversion to luminal A subtype assessed by PAM50 at surgery was evaluated in this study, a numerically higher percentage of conversion to luminal A subtype was observed in the letrozole plus ribociclib arm compared to the CT arm. Additionally, similar rates of low-ROR score at surgery were found in both arms.

- NEOLBC study(9) (NCT03283384): an ongoing randomized, multicenter, open-label, phase II clinical trial in n=100 postmenopausal patients with HR-positive HER2-negative, stages II/III BC. Based on Ki67 levels after two weeks of initial letrozole treatment, patients are advised to continue ET with letrozole (if Ki67 level < 1%) or to be randomized between standard CT [2-weekly plus G-CSF (granulocyte colony stimulating factor) followed by a taxane (3-weekly docetaxel or weekly paclitaxel)] vs. letrozole in combination with ribociclib (if Ki67 ≥ 1%). The aim of the study is to evaluate if CT could be replaced by the combination of letrozole plus ribociclib as a NA therapy for patients with non-metastatic primary luminal BC. The primary endpoint is to measure the difference in complete cell

cycle arrest (CCCA) defined as Ki67 < 1% determined by immunohistochemistry (IHC) between letrozole plus ribociclib vs. CT in the surgical specimen (around seven months after starting the initial treatment with letrozole) and to determine if letrozole plus ribociclib is associated to a ≥ 100% improvement in CCCA as compared to CT in the surgical specimen.

- PREDIX LumB study(10) (NCT02603679): an ongoing randomized phase II clinical trial in n = 200 luminal A/B BC patients with regional lymph node metastases, comparing NA weekly paclitaxel vs. standard ET plus palbociclib for 12 weeks; after 12 weeks treatment is switched crossover. Choice of ET is for pre- and perimenopausal women and all men tamoxifen, alternatively for women in this age cohort, a luteinizing hormone-releasing hormone (LHRH) analogue in combination with an aromatase inhibitor (AI), for all postmenopausal women treatment with an AI. During the 24-weekly treatment period, clinical and radiological evaluations are performed repeatedly. Switch between the treatment groups is allowed in case of lack of response or toxicity. Postoperatively, patients receive 3-weekly courses of CT with a combination of epirubicin and cyclophosphamide (EC). The primary endpoint is Radiological Objective Response Rate after completion of the first 12-week treatment period. Radiological response will be assessed by mammography and ultrasound, alternatively magnetic resonance imaging (MRI) of the breast could be used if mammography/ultrasound does not allow to perform objective measurements of the primary tumor. Clinical measurements using caliper is allowed if the tumor is palpable and the tumor size cannot be estimated by radiological methods.
- neoMONARCH study(61) (NCT02441946): assessed the biological effects of abemaciclib in combination with anastrozole in the neoadjuvant setting. Postmenopausal women with stage I–IIIB HR-positive/HER2-negative BC were randomized to a 2-week lead-in of abemaciclib, anastrozole, or abemaciclib plus anastrozole followed by 14 weeks of the combination. A total of 224 postmenopausal women with early-stage breast cancer were enrolled and randomly assigned to receive a 2-week lead-in of abemaciclib plus anastrozole (n = 74), abemaciclib monotherapy (n = 76), or anastrozole monotherapy (n = 74). The primary objective evaluated change in Ki67 from baseline to 2 weeks of treatment. Additional objectives included clinical, radiologic, and pathologic responses, safety, as well as gene expression changes related to cell proliferation and immune response. In total, 97% of patients completed cycle 1 (2 weeks of abemaciclib, anastrozole, or combination) and entered cycle 2, when all patients received abemaciclib plus anastrozole treatment for remainder of study treatment (14 weeks). Among patients who started cycle 2, 85% of patients completed study treatment (16 weeks) while 15% of patients discontinued study treatment for the following

reasons: AEs (n = 15), patient decision (n = 9), disease progression (n = 5), and other (n = 3). In addition, 23% of patients entered the extension period and received 8 additional weeks of study treatment. After treatment discontinuation, 85% of the intent-to-treat (ITT) population underwent definitive surgery.

Of the 224 ITT patients, baseline tumor Ki67 expression was available for 208 (93%) with Ki67 ≥ 5% in 195 (87%) of the tumors. Ki67 expression was assessed at baseline and after 2 weeks of treatment in tumors from 75% of patients including 80% in the combination arm, 69% in the abemaciclib arm, and 76% in the anastrozole arm. Ki67 expression decreased by a geometric mean change of −93% [90% confidence interval (CI), −95 to −90] in the combination arm, −91% (90% CI, −93 to −87) in the abemaciclib arm, and −63% (90% CI, −73 to −49) in the anastrozole alone arm. The geometric mean ratios for abemaciclib plus anastrozole versus anastrozole were 0.2 (90% CI, 0.1–0.3; P < 0.001) and 0.3 (90% CI, 0.2–0.4; P < 0.001) for abemaciclib versus anastrozole. Abemaciclib, alone or in combination with anastrozole, achieved a significant decrease in Ki67 expression and led to potent cell-cycle arrest after 2 weeks of treatment compared with anastrozole alone. In total, 47% of tumors demonstrated CCCA. More patients in the abemaciclib-containing arms vs. anastrozole alone achieved CCCA (58%/68% vs. 14%, P < 0.001). At the end of treatment, following 2 weeks lead-in and 14 weeks of combination therapy, 46% of ITT patients achieved a radiologic response, with pCR observed in 4%. Subgroup analyses did not demonstrate significant association between the abemaciclib-driven change in Ki67 expression at 2 weeks in any of the clinical, pathologic, and molecular subgroups.

Radiologic assessments at EOT, using the same method used at baseline, were available for 79% of patients. The radiologic ORR was 46% in the ITT population with 5% of patients having a CR and 42% a PR. Of the 190 patients who underwent surgery at EOT, 4% of patients had no evidence of invasive disease in the breast and axillary lymph nodes.

In patients with Ki67 analysis available at EOT, the decrease in Ki67 expression from baseline to EOT was similar regardless of the initial 2 weeks of therapy and a similar rate of CCCA was observed at EOT across all arms. To determine the extent to which abemaciclib-containing treatment suppresses tumor cell proliferation in high grade tumors, Ki67 expression at baseline, 2 weeks, and EOT as a function of tumor grade was examined (n = 138). Pathologic grade 2 and grade 3 tumors exhibited higher baseline Ki67 and most tumors, regardless of grade or baseline Ki67 expression, showed a reduction in Ki67 with treatment at 2 weeks, with a larger reduction observed in the abemaciclib-containing treatment arms compared to anastrozole alone.

PIK3CA mutation status was evaluable for 122 tumors; 36% of tumors harbored a PIK3CA-activating mutation. There were more PIK3CA mutant tumors in the

combination arm (32%) than in the abemaciclib (19%) and anastrozole (27%) arms. The presence of a PIK3CA mutation had no significant effect on Ki67 expression change from baseline to 2 weeks, including the rate of CCCA in response to abemaciclib alone (P = 0.570) or in combination compared to anastrozole (P = 0.216). It was also examined whether study treatment had any effect on the presence of tumor-infiltrating lymphocytes (TIL) in the tumor stroma. At baseline most of the tumors (88%, in combination arm; 92%, in abemaciclib arm; 94%, in anastrozole arm) had low TILs (0%–10% lymphoid cells) while a small subset (≤ 10%) of tumors had TILs in the 10% to 40%, and > 40% ranges. Treatment with abemaciclib, anastrozole, or abemaciclib plus anastrozole had no effect on the percentage of stroma TILs either at 2 weeks or 16 weeks (EOT). Treatment with the combination of abemaciclib plus anastrozole resulted in upregulation of the allograft rejection, inflammatory response, and IFNg response Hallmark gene sets at 2 weeks and EOT. Many other immune pathway gene sets were also enriched, including the PD-1 pathway gene set, highlighting a potential increase in the presence of activated T cells. Although these changes were consistent across early and late time points for the abemaciclib plus anastrozole combination arm, inflammatory immune gene changes were not significantly changed for those evaluable tumors treated with abemaciclib or anastrozole monotherapy during the 2-week lead-in period.

The most common all-grade AEs were diarrhea (62%), constipation (44%), and nausea (42%). Abemaciclib, anastrozole, and the combination inhibited cell-cycle processes and estrogen signaling; however, combination therapy resulted in increased cytokine signaling and adaptive immune response indicative of enhanced antigen presentation and activated T-cell phenotypes.

The poor pathological and biomarker responses of NACT achieved in NeoPAL study suggest that CDK4/6i might be and alternative, with a better safety profile, to CT in early high-risk BC. We hypothesized that some HR-positive/ HER2-negative breast tumors derive limited benefit from CT and might be treated with an exclusive endocrine-based CDK4/6i approach. In support of this there are results from ACOSGZ1031b study(13). In this study, tumors resistant to NAET (defined by Ki67 > 10% after two-week treatment with an AI) were also resistant to NACT. These highly proliferating endocrine resistant tumors treated with conventional CT had a 5% pCR rate, when a 20% pCR rate was expected. In agreement with this, resistant tumors in ACOSOGZ1031b were enriched in an E2F4 signature(14). This signature is prognostic of bad outcome and can be downregulated in patients by treatment with palbociclib. Interestingly, in BC cell lines resistant to estradiol deprivation, abemaciclib and palbociclib, but not paclitaxel nor fulvestrant, were able to completely suppress the expression of all genes composing the E2F4 signature(14).

In the NeoMONARCH study, abemaciclib, alone or in combination with anastrozole as NA in EBC, reduced Ki67 expression after 2 weeks of treatment based on geometric mean change and patients with complete cell cycle arrest (Ki67 < 2.7%)(13, 14):

- 78 patients achieved a Ki67 < 2.7%: 67.8% patients treated with abemaciclib + anastrozole, 57.7% patients treated with abemaciclib and 14.3% patients treated with anastrozole.
- The ORR by radiologic assessment at the completion of the study treatment (16 weeks) was of 46.4% and the pCR rate was of 3.7%.
- These data support continued evaluation of abemaciclib in EBC patients.

If MONARCH-E mirrors NeoMONARCH results, then MONARCH-E study will meet its primary end-point and abemaciclib will obtain the indication in the adjuvant setting for a high-risk patient population. Due to MONARCH-E inclusion criteria, abemaciclib is being explored mostly in a post-CT setting, so, one question MONARCH-E won´t answer is if abemaciclib could avoid the need of CT. This hypothesis can be efficiently tested in a neoadjuvant study, which can provide a go-no-go signal for an adjuvant trial and meaningful information regarding the target population.

Current NA studies are optimized regarding the CT duration (4-6 months), but not for CDK4/6i/ET duration. The optimal length of CDK4/6i/ET treatment in the neoadjuvant setting is unknown. So far, a maximum of 20 weeks has been explored but, as stated above, some patients might benefit of longer NAET and some mechanism of action of abemaciclib beyond cell cycle arrest, such as induction of senescence, immune cell recruitment and apoptosis(62), might be enhanced by a longer treatment duration. In fact, in the metastatic setting abemaciclib/ET achieves, in patients with measurable disease, an impressive 60% overall response rate, in the upper range of CT(33). Interestingly, the response rate increase with treatment duration (45% tumor reduction at 6 months vs. 64% at 12 months).

The fact that NAET achieves low pCR in ER-positive/HER2-negative BC but survival outcomes are relatively good, suggest that a biological biomarker might capture better the CDK4/6i mechanisms of action. In this regard, it would be worth exploring the ability of abemacilcib/ET to induce molecular downstaging detected through a validated genomic signature. Exploring the ability of abemacilcib/ET to decrease the risk score of a genomic signature with prognostic ability (such as the 21-gene Oncotype DX® Recurrence Score (RS)(20), the 70-gene MammaPrint®(21) or others(63)) would highlight the potential role of abemaciclib in improving the molecular state of patients who could subsequently skip post-surgery adjuvant CT.

Increasing evidence suggests that HR-positive/HER2-negative EBC is a heterogeneous disease(27) , and since the advent of genomic signatures, efforts have been made to integrate these signatures into clinical practice in the adjuvant setting for those patients with HR-positive/HER2-negative and pathological N0-1 BC.

WSG-PlanB (NCT01049425) is a prospective, randomized, multicenter phase III trial with CT in HER2-negative EBC. The planned translational analyses included the prospective comparison of the prognostic value of histological grade and IHC markers (ER, PR, Ki67 index, and IHC4) determined by independent central pathology with that of a genomic signature (Oncotype DX RS) in EBC(64). HR-positive/HER2-negative patients with Ki67 index levels ≥ 40% had poor survival, similar to that of TNBC patients. However, an unfavourable impact of higher Ki67 index on DFS was seen only in the RS > 25 subgroup of locally confirmed HR-positive patients, whereas no impact of Ki67 index was seen in corresponding RS ≥ 11 and RS 12–25 subgroups. Concordance between RS and Ki67 index risk assessment is relatively high in the Ki67 index ≤ 10% and ≥ 40% subgroups and the most prognostic value of RS was in the subgroup of intermediate Ki67 index (from > 10% to < 40%). Hence, using genomic signatures may be particularly useful for intermediate/high-risk pN0-1 disease according to clinical and/or IHC markers.

To sum up, the hypothesis we would like to test is if in intermediate-high risk BC patients, defined by TNM and IHC markers, the prolongation of NA abemaciclib + ET is non-inferior to standard NACT.

2. Objectives

2.1. Primary Objective

To assess the Residual Cancer Burden (RCB) 0-I rate in both treatment arms.

2.2. Primary End-point

Evaluation of the number of patients with a Residual Cancer Burden (RCB) 0-I index as a measure of efficacy. RCB(23) is a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden. It is estimated from routine pathological sections of the primary breast tumor site and the regional lymph nodes after the completion of NA therapy. The pathological variables include bidimensional diameters of the primary tumor bed, the proportion of primary tumor area containing invasive carcinoma, the number of positive lymph nodes, and the diameter of the largest nodal metastasis.

2.3. Secondary Objectives

- Changes in Ki67 index value after 2 weeks of treatment in both treatment arms.
- RCB 0+I vs. RCB-II vs. RCB-III in both treatment arms (TNM downstaging).
- Changes in RCB value between both treatment arms.
- Rate of PEPI score 0 at surgery in both treatment arms.
- Clinical response measured by magnetic resonance imaging (MRI) according to RECIST v1.1 in both treatment arms.
- Rate of breast conservative surgery (BCS) in both treatment arms.
- iEFS (invasive Event Free Survival) in both treatment arms.
- Assessment of safety profile by NCI-CTCAE v5.0 classification.
- To assess molecular downstaging for high risk genomic groups defined by a multigene expression panel.

2.4. Secondary End-points

- Percentage of decrease in the geometric mean of Ki67 index value after 2 weeks of treatment in both treatments arms.

  Number of patients with cell cycle arrest (Ki67 < 2.7%) after 2 weeks of treatment in both treatment arms.

- RCB is classified in four classes based on the residual disease (RD):

  - RCB-0 defined as pathological complete response.
  - RCB-I defined as minimal RD.
  - RCB-II defined as moderate RD.
  - RCB-III defined as extensive RD.
- Variation of the value of RCB based on the RD between the two treatment arms.
- PEPI(24) (Preoperative Endocrine Prognostic Index) requires pathological stage (tumor size and nodal status), level of Ki67 protein, and Allred ER score measured on the surgical specimen. PEPI score 0 includes pT1 or pT2, pN0, Ki67 $\leq$ 2.7%, Allred score > 2.
- Clinical Response Rate (CRR) is defined as the proportion of subjects with complete or partial radiographic response. CR and PR definitions are assessed by MRI at baseline and prior to breast surgery, with or without regional lymph nodes surgery, and categorized according to percent reduction in tumor size.
- Rate of breast conservative surgery (BCS): defined as the proportion of patients who achieved breast-conserving surgery between both treatment arms.
- Invasive event free survival (iEFS): defined as time from randomization to progressive disease or invasive disease recurrence (local, regional, distant, or contralateral), or death from any cause. Invasive disease recurrence is defined as:
  - Ipsilateral invasive breast tumor recurrence (including second primary invasive breast cancer): an invasive breast cancer involving the same breast parenchyma as the original primary lesion.
  - Ipsilateral regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, other regional lymph nodes, chest wall, and/or skin of the ipsilateral breast).
  - Distant recurrence (i.e., evidence of breast cancer in any anatomic site outside local and/or regional location and that has been either histologically confirmed or clinically diagnosed as recurrent invasive breast cancer)
  - Contralateral invasive breast cancer
  - Second primary invasive cancer of non-breast origin.
- Safety will be assessed by standard clinical and laboratory tests (haematology, serum chemistry). AEs grade will be defined by the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) version 5.0. AEs terms will be coded according to MedDRA dictionary.

- Gene expression data provided by a multigene expression panel in sequential tumor biopsies.

2.5. Exploratory Objectives

- Correlation of Ki67 protein level change after 2 weeks of treatment with some efficacy variables.
- Genetic changes in sequential tumor samples and tumor evolution during NA therapy, and its correlation with some efficacy variables and surrogate endpoints for response.
- To explore T-cell functional activation, immune suppression, neoantigens and cytokines production as well as other immune response biomarkers (such as CD4/CD8/FOXP3/PDL-1), in order to understand the activity of abemaciclib on the tumor microenvironment and the immune response.
- Circulating tumor DNA (ctDNA) dynamics as a surrogate endpoint for response and prognostic implications.
- Sequential genomic profiling and quantification of ctDNA samples to explore early response dynamics, minimal residual disease, tumor tracking, tumor mutational burden and clonal diversity along NA therapy and treatment follow up.
- To investigate if ctDNA dynamics after 2-3 weeks of treatment predicts PEPI score, molecular downstaging, and minimal residual disease in ctDNA samples, and its correlation with some efficacy variables.
- To investigate if ctDNA dynamics after 3-4 weeks post-surgery predicts PEPI score and molecular downstaging, and its correlation with some efficacy variables.
- Genetic changes in pre vs. post NA therapy tumor samples to identify potential genomic mechanisms of resistance.
- To describe ctDNA mutation landscape in resistant tumors.
- Generation and expansion of ex vivo organoid-based models and/or xenographs from post-therapy tumors and if possible, from baseline tumors to perform high-throughput functional screening studies.
- Identification of indirect biomarkers of tumor biology and treatment activity by metabolomic analysis (glutamine pathway).
- To explore other possible biomarkers of clinical activity in tumor and blood samples.

2.6. Exploratory End-points

- Variation of the Ki67 levels, determined by immunohistochemistry (IHC), between pre-treatment and after 2 weeks-treatment biopsies, and its correlation with some efficacy variables.
- Mutational and copy number variation (CNV) data analyzed in pre-treatment and surgery tumor samples (large targeted gene panels including TP53 and MYC mutations and MYC CNVs) and its correlation with some efficacy variables.
- Gene expression data provided by a multigene expression panel in sequential tumor biopsies and its correlation with some efficacy variables.
- Monitoring of ctDNA abundance and specific tumoral mutation and genetic changes observed in ctDNA samples along treatment and follow up.
- Mutations and CNV detected in ctDNA collected after recurrence.
- Values of other proteins, metabolites, RNA or DNA alterations obtained from the tissue or blood samples and its correlation with efficacy variables could be used for assessment of biomarkers related to activity of abemaciclib and breast tumor sensitivity and/or resistance to treatment.

3. Investigational Plan

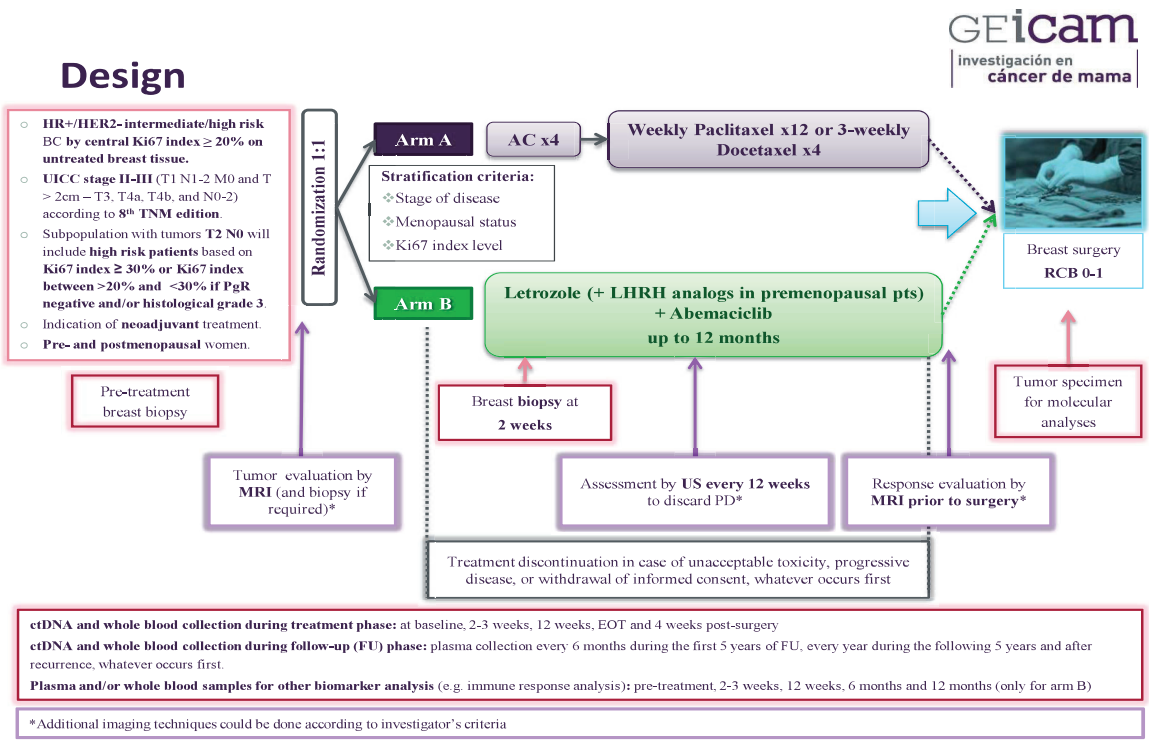
3.1. Study Design

This is an international, multicenter, open-label, randomized phase II study in the NA setting. Approximately 200 pre- and postmenopausal women with HR-positive/HER2-negative intermediate/high risk BC determined by central Ki67 index ≥ 20% on untreated breast tissue, with indication of NA treatment, will be included.

Once the screening process (local at site and at the central laboratory) is completed, fully eligible patients will be randomized in a 1:1 fashion to the control arm with standard chemotherapy based on anthracyclines and taxanes or to the experimental arm with Letrozole + Abemaciclib (+ LHRH analogs in premenopausal women). Patients will be stratified according to the disease stage (II vs. III), menopausal status (premenopausal vs. postmenopausal) and Ki67 index (Ki67 < 30% vs. Ki67 ≥ 30%). All patients will be treated according to the stipulations below, unless any of the following occur: unacceptable toxicity, progressive disease (PD), or withdrawal of informed consent, whatever occurs first.

After the last dose of any of the drugs in the NA combinations, in both treatment arms definitive surgery will be performed. For Arm A not earlier than 21 days and not later than 42 days after the last dose of chemotherapy, and for Arm B within 7 days from the last dose of abemaciclib and/or letrozole, unless toxicities are not recovered completely in any treatment arm.

Figure 1. Study Design



Control Arm (Arm A):

- Doxorubicin 60 mg/m^2 and Cyclophosphamide 600 mg/m^2 (AC) every 21 days for 4 cycles followed by
- weekly paclitaxel 80 mg/m^2 for 12 weeks or 3-weekly docetaxel 100 mg/m^2 for 4 cycles.

Approximately duration of 24 weeks (6 months).

In Arm A, luteinizing hormone-releasing hormone (LHRH) analogs are not allowed in premenopausal women.

Experimental Arm (Arm B):

- Letrozole 2.5 mg orally daily + abemaciclib 150 mg orally every 12 hours on a continuous dosing schedule, plus LHRH analogs in premenopausal women, up to 12 months (±14 days), with study visits each 28 (±3) days from first study dose.

Schedule of study procedures is provided in Protocol Attachment 1. Study Schedule.

In order to discard metastases, the following tests should be performed before randomization: bone scan if bone pain and/or elevated alkaline phosphatase; abdominal/pelvic computerized tomography scan (CT) if elevated alkaline phosphatase,

abnormal liver function tests, abdominal symptoms or abnormal physical examination; chest CT scan if pulmonary symptoms.

A serum pregnancy test should be done within 7 days of the first dose of study treatment in both arms for Child Bearing Potencial (CBP) patients.

Once the screening process (local at site and at the central laboratory) is completed, fully eligible patients will be randomized in a 1:1 manner. Tumor evaluation will be done by MRI in baseline and prior to breast surgery, and US will be repeated every 12 week (+/- 1 week) during the treatment phase to discard PD, as scheduled according to the calendar regardless of any dosing delay.

For Arm B MRI could be done from 11 months of study treatment, to allow enough time to plan surgery within 7 days of the last dose of abemaciclib and/or letrozole.

During treatment phase a serum or urine pregnancy test must be performed in every cycle and at the post-treatment visit for CBP patients, including women with tubal ligation.

After NA treatment in each arm, patients will undergo breast surgery, with or without regional lymph nodes surgery, of the primary breast cancer. Adjuvant CT will be allowed as per investigator's judgment based on the assessment of the pathological tumor response.

Those patients who do not complete the NA treatment according to the study protocol because of progression of the disease (PD), unacceptable toxicity or informed consent withdrawal, will be discontinued from the study treatment and treated as per investigator's judgment and only survival follow-up and death dates (if applicable) will be collected.

Additionally, in those patients who experience recurrence during the follow-up period of the study, survival follow-up will also be collected.

For survival follow-up, only dates on which the patient is alive or not will be collected.

Patients enrolled on the study will be followed for a period of 10 years.

For specific details regarding all study procedures, please see Protocol Attachment 1. Study Schedule.

3.2. Duration of the study

It is estimated that the enrollment will be completed approximately in 24 months.

Following the randomization, patients will receive the treatment for approximately 6 months in Arm A and 12 months (±14 days) in Arm B, unless PD, unacceptable toxicity or withdrawal of the informed consent, whatever occurs first. After treatment the patient will undergo surgery. After surgery, there will be follow-up of 10 years.

For safety reasons all patients will have a visit after finishing treatment with the study medications.

In **Arm A** patients will have this visit not earlier than 21 days after finishing treatment with the study medications and before definitive surgery. In **Arm B** the visit will be done within 7 days from the last abemaciclib and/or letrozole dose.

In some patients, the end of the study treatment (at neoadjuvant setting) may be due to PD or toxicity. In case the beginning of the new therapy cannot be delayed as per the investigator's judgment, the safety visit may be performed in advance and always before starting the new anticancer therapy.

The start date of study is the date of the first site activation.

The end date of study is the date of the last visit of the last patient (LPLV) including follow up. Performing exploratory analyses will be independent of the date of the end of the study.

4. Study Population

4.1. Inclusion Criteria

Patients are eligible to be enrolled in the study only if they **meet all** of the following criteria:

1. Written informed consent prior to any specific study procedures.
2. Women ≥ 18 years of age.
3. Documentation of histologically confirmed primary invasive adenocarcinoma of the breast. Adenocarcinoma with another component of epithelial origin (for example, medullary or neuroendocrine) is allowed.
4. Availability of a primary tumor tissue sample obtained during the diagnostic process before treatment for the central Ki67 index and biomarker exploratory analyses (following the specifications described in the Sample Management Manual of the study).
5. Documentation of HR positive and HER2 negative BC based on local laboratory determination.
    a. HR positive is defined as more than or equal to 10% positive cells by immunohistochemistry (IHC) for ER and/or progesterone receptor (PgR).
    b. HER2 negative tumor is determined according to recommendations of ASCO/CAP 2018 guidelines.
6. Intermediate and high risk patients based on Ki67 index value (≥ 20%) determined at a central laboratory.
7. Patients should be in the following clinical stages of disease according to the 8th edition of the TNM Classification of Breast Cancer, by the UICC (Union for International Cancer Control): T1c N1-2 M0 and T2 (> 2cm) – T3, T4a, T4b, N0 – N2, M0 (stages IIA, IIB, IIIA or IIIB). Subpopulation with tumors T2 N0 will include high risk patients based on Ki67 index ≥ 30% or Ki67 index between ≥ 20% and <30% if PgR negative and/or histological grade 3.
8. Patients with diagnosis of suspicious for multifocal or multicentric breast cancer will be eligible for the study. At least two tumor lesions should be biopsied. All histologically available tumor lesions must comply with the inclusion criteria no. 5.
    a. If all lesions have similar morphological characteristics (i.e. based on local assessment of type, grade, Ki67 index level, etc.…), only the largest tumor lesion will be assessed for central assessment of Ki67 index level.

b. If lesions have different morphological characteristics, discordant tumor lesions will be centrally evaluated for Ki67 index level. Patients will be eligible if at least one tumor lesion complies with criteria 6 and 7.

9. Indication of NA therapy.
10. At the time of presentation, patients must be candidates for potentially curative surgery by surgeon's assessment.
11. Sentinel lymph node biopsy (SLNB) will be preferable after the NA treatment. Those patients with SLNB before the NA treatment will be eligible for the study only if the SLNB has a negative result (N0). One Step Nucleic Acid Amplification (OSNA) method is not allowed.
12. Pre- and postmenopausal women.

    Postmenopausal status is defined as:

    - Patient underwent bilateral oophorectomy, or
    - Age ≥ 60 years, or
    - Age < 60 years and amenorrhea for 12 or more months (in the absence of chemotherapy, tamoxifen, toremifene or ovarian suppression) and Follicle-stimulating hormone (FSH) and plasma estradiol are in the postmenopausal ranges per local normal ranges.

    All women who do not meet the criteria for postmenopausal status are considered premenopausal for the purpose of this trial.
13. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1.
14. Patients are able to swallow oral medications.
15. Adequate organ and bone marrow function:

    - ANC ≥ 1,500/mm^3 (1.5x10^9/L);
    - Platelets ≥ 100,000/mm^3 (100x10^9/L);
    - Haemoglobin (Hgb) ≥ 8g/dL (80g/L) (erythrocyte transfusions are permited; initial treatment must not begin earlier than the day after the erythrocyte transfusion);
    - Total serum bilirubin ≤ 1.5xULN (≤2xULN and direct bilirubin within normal limits if Gilbert´s disease);
    - AST and ALT ≤ 3xULN.

16. Left ventricular ejection fraction (LVEF) ≥ 50% measured by multiple-gated acquisition scan (MUGA) or echocardiogram (ECHO).
17. For premenopausal women: agreement to remain abstinent or use single or combined non-hormonal contraceptive methods that result in a failure rate of < 1% per year during the treatment period and for at least 3 weeks after the last dose of study treatment. Abstinence is only acceptable if it is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.

    Examples of non-hormonal contraceptive methods with a failure rate of < 1% per year include tubal ligation, male sterilization, and certain intrauterine devices. Alternatively, two methods (e.g., two barrier methods such as a condom and a cervical cap) may be combined to achieve a failure rate of < 1% per year. Barrier methods must always be supplemented with the use of a spermicide.
18. Negative serum pregnancy test within 7 days of the first dose of abemaciclib or chemotherapy for premenopausal women, and for women who have experienced menopause onset < 12 month prior to first dose of therapy.
19. Patients consent to biological sample provision for biomarker exploratory analyses.
20. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

4.2. Exclusion Criteria

Patients will be excluded from the study if they **meet any** of the following criteria:

1. Previous anti-cancer treatment with therapeutic intent for current breast cancer is not allowed.
2. Patients with inflammatory breast cancer or synchronous bilateral invasive breast cancers are not eligible.
3. Serious and/or uncontrolled preexisting medical condition(s) that in the judgment of the investigator, would preclude participation in this study (for example, interstitial lung disease, severe dyspnea at rest or requiring oxygen therapy, severe renal impairment [e.g. estimated creatinine clearance < 30ml/min], history of major surgical resection involving the stomach or small bowel, or preexisting Crohn's disease or ulcerative colitis or a preexisting chronic condition resulting in baseline Grade 2 or higher diarrhea).
4. Patients with rare hereditary problems of galactose intolerance, total lactase deficiency or glucose- galactose malabsorption.

5. Females who are pregnant or lactating.
6. Active systemic bacterial infection (requiring intravenous [IV] antibiotics at time of initiating study treatment), fungal infection, or detectable viral infection (such as known human immunodeficiency virus positivity or with known active hepatitis B or C [for example, hepatitis B surface antigen positive]. Screening is not required for enrollment.
7. Personal history of any of the following conditions: syncope of cardiovascular etiology, ventricular arrhythmia of pathological origin (including, but not limited to, ventricular tachycardia and ventricular fibrillation), or sudden cardiac arrest.
8. Diagnosis of any other malignancy within 5 years prior to randomization, except for adequately treated basal cell or squamous cell skin cancer, or carcinoma in situ of the cervix, breast or colorectal.
9. Prior hematopoietic stem cell or bone marrow transplantation.
10. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

4.3. Discontinuations

4.3.1. Discontinuation of Study Treatment

The criteria for enrollment must be followed explicitly. If a patient who does not meet enrollment criteria is inadvertently enrolled, that patient should be discontinued from the study treatment, but can be allowed to continue in the study in order to provide the follow-up data needed for the analysis of the entire population. An exception may be granted if the patient, in the opinion of the investigator, is having benefit from the study treatment. In these rare cases, the investigator must obtain documented approval from GEICAM to allow the patient to continue receiving the study treatment provided by the Sponsor.

Patients can be discontinued from the study therapy in the following circumstances:

- Patient’s own request.
- Unacceptable toxicity as defined in the protocol.
- Tumor progression as defined in RECIST v1.1. In this case, the patient will be continued only for survival follow-up date.

- Any clinical adverse event (AE), laboratory abnormality or inter-current illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the patient.
- Pregnancy:
  - ✓ Instruct to contact the investigator or study staff immediately if they suspect they might be pregnant.
  - ✓ The investigator must immediately notify GEICAM if a study patient becomes pregnant.
- Termination of the study by GEICAM.
- Physician's decision, including need of other anti-cancer therapy, not specified in the protocol.
- If the patient is non-compliant with study procedures.
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g. infectious disease) illness.

All permanent treatment discontinuation should be recorded by the Investigator in the eCRF when considered as confirmed.

4.3.2. Discontinuation of Study Sites

Study Site participation may be discontinued if GEICAM, the investigator or the EC/IRB of the study site judges it necessary for any reason.

4.3.3. Discontinuation of Study

The study may be discontinued by GEICAM if this is medically reasonable and consistent with applicable regulations of Good Clinical Practice (GCP). Stopping the study for medical reasons may be required if patients experienced adverse reactions under the study treatment or if new information about the safety or effectiveness of the study treatment justifies it.

5. Treatment

5.1. Treatments Administered

5.1.1. Study Treatment Arm A

Control Arm (Arm A):

- Doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 (AC) every 21 days for 4 cycles followed by
- weekly paclitaxel 80 mg/m^2 for 12 weeks **or** 3-weekly docetaxel 100 mg/m^2 for 4 cycles.

Approximately duration of 24 weeks (6 months).

In Arm A, luteinizing hormone-releasing hormone (LHRH) analogs are not allowed in premenopausal women.

If the patient receives weekly paclitaxel 80 mg/m^2 for 12 weeks, hematology, biochemistry and the hospital visits can be performed every 21 days (each 3 week paclitaxel administration corresponds to one cycle).

The real Body Surface Area (BSA) of the patient determined in the baseline visit will be the reference BSA throughout the study. The doses will be recalculated in the event that patients experience body weight variations greater than 10% during the treatment period.

5.1.2. Study Treatment Arm B

Experimental Arm (Arm B):

- Letrozole 2.5 mg orally daily + abemaciclib 150 mg orally every 12 hours on a continuous dosing schedule, (+ LHRH analogs in premenopausal women), up to 12 months (±14 days), with study visits each 28 (±3) days from first study dose.

The beginning of LHRH analogs administration will be as per investigator's judgment.

5.2. Materials and Supplies

The definition of Investigational Medicinal Product (IMP) is a drug that is being studied or used as a reference, even as placebo, in the context of a clinical trial, regardless of its authorization.

All IMPs used in the trial will be named as study treatment along the protocol.

Abemaciclib will be provided for free to the study sites by the Sponsor with the appropriate label for clinical trial use for the purpose of this study.

Letrozole, doxorubicin, cyclophosphamide and paclitaxel or docetaxel are marketed products and will be procured according to local practice and regulation.

5.2.1. Storage, preparation and administration

Investigators and site staff are reminded to continuously monitor temperatures and ensure that thermometers are working correctly as required for proper storage of study treatment.

Abemaciclib should be stored at controlled room temperature in their original container, as specified on the drug labels and in the summary of product characteristics (SmPC).

For abemaciclib provided by the Sponsor, any temperature excursions must be reported immediately to GEICAM and documented. Once a deviation is identified, the study treatment MUST be quarantined and not used until GEICAM provides documentation of permission to use the study treatment product.

Abemaciclib should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned study treatment provided by the Sponsor should be stored separately from study treatment that needs to be dispensed.

Storage area temperature conditions must be monitored and recorded daily. A daily temperature log will also be kept at the study site.

Refer to the current SmPC for details regarding the storage and handling of letrozole, doxorubicin, cyclophosphamide and paclitaxel or docetaxel. Any issue or deviation that affect the storage conditions or handling of letrozole, doxorubicin, cyclophosphamide and paclitaxel or docetaxel must be reported to the Sponsor for its information.

5.2.1.1. Abemaciclib

Abemaciclib will be supplied as bottles with 60 film-coated tablets containing 50mg of abemaciclib.

Initially dose will be 150mg twice a day = 6 tabs/day or 3 bottles/cycle (28 days cycle).

Abemaciclib should be taken twice a day continuously for 12 months (±14 days) or until unacceptable toxicity occurs.The dose can be taken with or without food. It should not be taken with grapefruits or grapefruit juice.

Patients should take the doses at approximately the same time every day, preferable in the morning and evening.

The tablet should be swallowed whole, with a glass of water (patients should not chew, crush, or spit tablets before swallowing).

If a patient vomits or misses a dose of abemaciclib, the patient should be instructed to take the next dose as its scheduled time; an additional dose should not be taken.

The patient number should be recorded on the label in the spaces provided by site personnel at the time of assignment to patient. Site personnel must ensure that patients clearly understand the guidelines for self-medication. Patients should be given a sufficient supply to last until their next study visit. Unused drug and/or empty bottles should be returned to the site at the next study visit. Returned unused medication MUST NOT be re-dispensed to patient.

At each visit patient will be dispensed the number of bottles necessary to complete the cycle, according the dose prescribed. In the event of dose modification, request should be made of the patient to return all previously dispensed medication to the clinic and new bottles will be dispensed.

Patients experiencing investigational product related toxicity may have their dose modified according to Section 5.4.1.

5.2.1.2. Letrozole

Letrozole should be taken orally and can be taken with or without food.

A missed dose should be taken as soon as the patient remembers. However, if it is almost time for the next dose (within 2 or 3 hours), the missed dose should be skipped, and the patient should go back to her regular dosage schedule. Doses should not be doubled because with daily doses over the 2.5mg recommended dose, over-proportionality in systemic exposure was observed.

Please, refer to the current SmPC for further details.

5.2.1.3. Doxorubicin, Cyclophosphamide and Paclitaxel or Docetaxel

Investigators should consult the locally approved prescribing information for storage and administration instructions.

5.2.2. Accountability

It is the responsibility of the investigator to ensure that a current record of abemaciclib disposition is maintained at each study site where study treatment provided by the Sponsor are inventoried and disposed.

A drug dispensing log, including records of drug received from the Sponsor will be provided by GEICAM if the staff at the investigational site does not have an established system that meets these requirements, and will be kept at the study site. Records or logs must comply with applicable regulations and guidelines.

On a per-patient basis, records must be maintained documenting dates and quantities (ie, pill counts) of provided study treatment dispensed and returned at each study visit. Any

study treatment provided by the Sponsor accidentally or deliberately destroyed must be documented.

Abemaciclib provided by the Sponsor will be preferably destroyed at each participating site. The site must obtain written authorization from the Sponsor before it is destroyed, and this destruction must be documented on the appropriate form.

It is also the responsibility of the investigator to ensure that current records of letrozole, doxorubicin, cyclophosphamide and paclitaxel or docetaxel dispensations are available to assure traceability.

5.3. Method of Assigning a Patient to a Treatment

Patients will be screened by one of the investigators prior to study entry. An explanation of the study and discussion of the expected side effects and presentation of the informed consent document will take place. Eligible and consented patients will be screened and then enrolled into the study.

All patients screened in the study, will be included in a *Patient Screening Log* maintained at each site and at the GEICAM central office.

No patients can be enrolled and receive study treatment until the patient has been screened in the study. All eligibility criteria must be met at the time of enrollment (not necessary in screening failure patient). There will be no exceptions. Any question should be addressed with GEICAM prior to enrollment. An eligibility checklist must be completed and signed by the Principal Investigator or Sub-Investigator before enrolling each patient to confirm all inclusion/exclusion criteria and stratifications factors. This eligibility checklist should be filed with the study documentation. Once the eligibility checklist is completed, the study personnel at the site will enroll the patient through the eCRF and the system will send the unique randomization number of the patient and the assigned arm to the site. This randomization number should be used on all documentation and correspondence with the GEICAM central office. Only after the confirmation of enrollment by the system, the patient can receive the study treatments. All patients enrolled in the study will be registered in a *Patient Enrollment and Identification Log* that will be only maintened at the site.

Study treatment(s), must be administered within 5 days from enrollment.

5.4. Special Treatment Considerations. Dose Adjustments of Study Treatment

All dose modifications should be based on the worst preceding toxicity.

Every effort should be made to administer study treatment at the planned dose and schedule. However, in the event of significant treatment-related toxicity, administration of

study treatment may need to be adjusted as described in the following sections. Depending on the nature of the toxicity observed, dose adjustments may be required for one or more study treatment in the combination.

All dose modifications/adjustments must be clearly documented in the patient's source notes and the appropriate section of the eCRF.

Control Arm (Arm A):

If a treatment-related toxicity has not been recovered as the study protocol states after 1 complete cycle period of dose interruption or delay, permanent discontinuation of study treatment may be considered. Treatment resumption for patients recovering from treatment-related toxicity after > 1 cycle of treatment interruption or cycle delay, but deemed to be deriving obvious clinical benefit per the investigator's best medical judgment, should be discussed with GEICAM.

In the event that the start of a new cycle is delayed due to treatment-related toxicity, procedures required on day 1 of the given cycle will be performed when medication is resumed. New cycle day 1 procedures (ie. physical examination, ECOG performance status, blood chemistry, hematology) that were performed prior to knowing the need to delay the start of the cycle do not need to be repeated (1) if not required to determine whether study drug may be resumed and (2) if performed within 7 days prior to study drug resumption.

Experimental Arm (Arm B):

If a treatment-related toxicity has not been recovered as the study protocol states after 1 month of dose interruption, permanent discontinuation of study treatment may be considered. Treatment resumption for patients recovering from treatment-related toxicity after > 1 month of treatment interruption, but deemed to be deriving obvious clinical benefit per the investigator's best medical judgment, should be discussed with GEICAM.

Each study visit and procedure (ie. physical examination, ECOG performance status, blood chemistry, hematology) will be performed as scheduled according to the calendar regardless of any dosing omission and/or interruption, every 28 (± 3) days from the start of treatment.

5.4.1. Abemaciclib

In the event of significant treatment-related toxicity, abemaciclib dosing may be interrupted or delayed and/or reduced. Patients are to be instructed to notify investigators at the first occurrence of any adverse sign or symptom.

5.4.1.1. Dose Adjustements

Management of some adverse reactions may require dose interruption and/or dose reduction. Dose level reductions should be made in 50mg decrements. For example, if the starting dose of abemaciclib for a study is 150mg every 12 hours, dose reduction 1 would be 100mg every 12 hours, dose reduction 2 would be 50mg every12 hours. Please see **Table 2.**

Table 2. Dose adjustment recommendations for adverse reactions

	Abemaciclib dose combination therapy
Recommended dose	**150mg twice daily**
First dose adjustment	**100mg twice daily**
Second dose adjustment	**50mg twice daily**

Once a dose has been reduced for a given patient, all subsequent doses should be administered at that dose level, unless further dose reduction is required. Dose re-escalation is not allowed.

5.4.1.2 Guidelines for Diarrhea Management

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

- At the first sign of loose stools, the patient should initiate antidiarrheal therapy (e.g. loperamide) and notify the investigator for further instructions and appropriate follow-up.
- Patients should also be encouraged to drink fluids (e.g., 8 to 10 glasses of clear liquids per day).
- Site personnel should assess response within 24 hours. Guidance for Grade 2 diarrhea is to wait for 24h to see if diarrhea goes down to grade 1 or gets solved.

Refer to Table 3 for additional information for diarrhea management and dose modification.

Table 3: Dose Modification and Management- Diarrhea

At the first sign of loose stools, start treatment with antidiarrheal agents, such as loperamide.

CTCAE Grade	Abemaciclib Dose Modifications
Grade 1	No dose modification is required.
Grade 2	If toxicity does not resolve within 24 hours to ≤ Grade 1, suspend dose until resolution. Dose reduction is not required
Grade 2 that persists[a], or recurs[b] after resuming the same dose despite maximal supportive measures	Suspend dose until toxicity resolves to ≤ Grade 1 Resume at next lower dose
Grade 3 or 4 or requires hospitalization	

[a] Determination of persistent events will be at the discretion of the investigator.

[b] Please note that recurrent toxicity refers to the same event occurring within the next 8 weeks (as measured from the stop date of the preceding event).

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

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

5.4.1.4 General Guidance for Interstitial Lung Disease (ILD)/Pneumonitis Events

Interstitial lung disease (ILD) / pneumonitis has been identified as an adverse drug reaction (ADR) for abemaciclib. The majority of events observed in clinical trials were Grade 1 or Grade 2 with serious cases and fatal events reported. Additional information is available in the Investigator Brochure (IB).

Monitor for clinical symptoms or radiological changes indicative of ILD/pneumonitis and ask your patients to report any new or worsening pulmonary symptoms such as dyspnoea, cough and fever; these symptoms should be investigated and treated as per local clinical practice and/or guidelines (including corticosteroids as appropriate). If ILD/pneumonitis is suspected, investigations may include imaging such as high resolution computer tomography (HRCT), broncheoalveolar lavage (BAL), and biopsy as clinically indicated.

For patients who develop radiological changes suggestive of pneumonitis and have few or no symptoms (Grade 1), abemaciclib should be continued without dose modification. For

persistent or recurrent Grade 2 ILD/pneumonitis events, abemaciclib should be suspended until toxicity resolves to baseline or Grade 1, and resumed at the next lower dose. For ≥ Grade 3 ILD/pneumonitis events, permanently discontinue abemaciclib (see also **Table 8**: ***refer to dose adjustment table for non-hematological toxicities***).

5.4.1.5 General Guidance for Hepatic Monitoring

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) elevation are considered as ADR with the use of abemaciclib, and should be monitored according to **Table 4 and Table 5,** respectively.

Table 4: Dose Modification and Management — Increased ALT (GPT/SGPT) / AST (GOT/SGOT)

Monitor ALT and/or AST prior to the start of abemaciclib therapy, every 2 weeks for the first 2 months, monthly for the next 10 months, and as clinically indicated.	
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 Grade 2 that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1, or Recurrent[a] Grade 2 or Grade 3 (> 5.0-20.0 x ULN)	Suspend dose until toxicity resolves to baseline or Grade 1 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

[a] Please note that recurrent toxicity refers to the same event occurring within the next 8 weeks (as measured from the stop date of the preceding event).

To ensure patient safety the investigator should collect specific recommended clinical information and follow-up laboratory tests as shown below in **Table 5.**

Details for hepatic monitoring depend upon the severity and persistence of observed laboratory test abnormalities. If a study patient experiences elevated ALT and/or AST 5×ULN and elevated TBL 2×ULN, or ALT and/or AST 8×ULN, liver tests, including ALT, AST, TBL, direct bilirubin, gamma-glutamyl transferase (GGT), and creatine

phosphokinase (CPK), should be repeated within 3 to 5 days to confirm the abnormality and to determine if it is increasing or decreasing. If the abnormality persists or worsens, clinical and laboratory monitoring should be initiated by the investigator, based on the hepatic monitoring tests below.

Table 5: Hepatic Monitoring Tests for a Hepatic Treatment Emergent Abnormality

Hematology	**Clinical Chemistry**
Hemoglobin	Total bilirubin
Hematocrit	Direct bilirubin
Erythrocytes (RBCs - red blood cells)	Alkaline phosphatase (ALP)
Leukocytes (WBCs - white blood cells)	Alanine aminotransferase (ALT)
Differential:	Aspartate aminotransferase (AST)
Neutrophils, segmented	Gamma-glutamyl transferase (GGT)
Lymphocytes	Creatine kinase (CK)
Monocytes	**Other Chemistry**
Basophils	Acetaminophen
Eosinophils	Acetaminophen protein adducts
Platelets	Alkaline phosphatase isoenzymes
Cell morphology (RBC and WBC)	Ceruloplasmin
Coagulation	Copper
	Ethyl alcohol (EtOH)
Prothrombin time, INR (PT-INR)	Haptoglobin
Serology	Immunoglobulin IgA (quantitative)
Hepatitis A virus (HAV) testing:	Immunoglobulin IgG (quantitative)
HAV total antibody	Immunoglobulin IgM (quantitative)
HAV IgM antibody	Phosphatidylethanol (PEth)
Hepatis B virus (HBV) testing:	**Urine Chemistry**
Hepatitis B surface antigen (HBsAg)	Drug screen
Hepatitis B surface antibody (anti-HBs)	Ethyl glucuronide (EtG)
Hepatitis B core total antibody (anti-HBc)	**Other Serology**
Hepatitis B core IgM antibody	Anti-nuclear antibody (ANA)
Hepatitis B core IgG antibody	Anti-smooth muscle antibody (ASMA) [a]
HBV DNA [c]	Anti-actin antibody [b]
Hepatis C virus (HCV) testing:	Epstein-Barr virus (EBV) testing:
HCV antibody	EBV antibody
HCV RNA [c]	EBV DNA [c]
Hepatitis D virus (HDV) testing:	Cytomegalovirus (CMV) testing:
HDV antibody	CMV antibody
Hepatitis E virus (HEV) testing:	CMV DNA [c]

HEV IgG antibody	Herpes simplex virus (HSV) testing:
HEV IgM antibody	HSV (Type 1 and 2) antibody
HEV RNA [c]	HSV (Type 1 and 2) DNA [c]
Microbiology	Liver kidney microsomal type 1 (LKM-1) antibody
Culture:	
Blood	
Urine	

[a] Not required if anti-actin antibody is tested.

[b] Not required if anti-smooth muscle antibody (ASMA) is tested.

[c] Reflex/confirmation dependent on regulatory requirements, testing availability, or both.

5.4.1.6 General Guidance for Hematology Toxicity and Dose Modification

Hematologic toxicities including neutropenia, leukopenia, anemia, and thrombocytopenia have been observed in patients treated with abemaciclib, and causality has been established. Severe (Grade 3 and 4) neutropenia was observed in patients receiving abemaciclib. Patients should be monitored closely for signs of infection, anemia, and bleeding. The following instructions on **Table 6** must be followed:

Table 6: Dose Modification and Management — Hematologic Toxicities

Monitor complete blood counts prior to the start of abemaciclib therapy, every 2 weeks for the first 2 months, monthly for the next 10 months, and as clinically indicated.	
CTCAE Grade	**Abemaciclib Dose Modifications**
Grade 1 or 2	No dose modification is required
Grade 3	Suspend dose until toxicity resolves to ≤ Grade 2 Dose reduction is not required
Recurrent[b] Grade 3 or Grade 4	Suspend dose until toxicity resolves to ≤ Grade 2 Resume at next lower dose
Patient requires administration of a blood cell growth factor	Suspend abemaciclib dose for at least 48 hours after the last dose of blood cell growth factor and until toxicity resolves to ≤ Grade 2 Resume abemaciclib at next lower dose unless the dose was already reduced for the toxicity that led to the use of

	the growth factor

[b] Please note that recurrent toxicity refers to the same event occurring within the next 8 weeks (as measured from the stop date of the preceding event).

5.4.1.7 General Guidance for Venous Thromboembolic Events (VTE)

VTE has been identified as an adverse drug reaction (ADR) for abemaciclib in combination with ET. However, in studies with single-agent abemaciclib use in the metastatic BC or other tumor types, including non-small cell lung cancer (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 (DVT) and pulmonary embolism (PE) and treat as medically appropriate.

Table 7: Dose Modification and Management — Venous Thromboembolic Events

CTCAE Grade	Abemaciclib Dose Modifications
Grade 1 or 2	Suspend dose and treat as clinically indicated. Abemaciclib may be resumed when the patient is clinically stable.
Grade 3 or 4	Suspend dose and treat as clinically indicated. Abemaciclib may be resumed when the patient is clinically stable.

5.4.1.7 General Guidance for Non-hematologic Toxicities (excluding diarrhea, increased ALT/AST and Venous Thromboembolic Events) Monitoring

Non-hematologic toxicities excluding diarrhea and increased ALT/AST:

- Grade 1 or 2: no dose modification is required.
- Persistent or recurrent Grade 2 toxicity that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1: suspend dose until toxicity resolves to baseline or Grade 1. Resume at next lower dose.
- Grade 3 or 4: suspend dose until toxicity resolves to baseline or Grade 1. Resume at next lower dose. Permanently discontinue abemaciclib in case of Grade 3 or 4 ILD/pneumonitis.

Table 8: Dose Modification and Management — Nonhematologic Toxicities Excluding Diarrhea, ALT/AST Increased, and Venous Thromboembolic Events

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

[a] Please note that recurrent toxicity refers to the same event occurring within the next 8 weeks (as measured from the stop date of the preceding event).

[b] Permanently discontinue abemaciclib in case of Grade 3 or 4 ILD/pneumonitis.

Patients discontinuing abemaciclib treatment permanently due to treatment related toxicity will continued with letrozole in the active treatment phase of the study.

5.4.2. Letrozole

The most frequently reported adverse reactions in clinical studies with letrozole were hot flushes, hypercholesterolaemia, arthralgia, fatigue, increased sweating and nausea.

Important additional adverse reactions that may occur with letrozole are: skeletal events such as osteoporosis and/or bone fractures and cardiovascular events (including cerebrovascular and thromboembolic events).

No dose adjustment is required. Treatment interruption for letrozole-related toxicities will be performed as per the investigator´s best medical judgment.

In the event of a toxicity requiring dosing delay of abemaciclib, the administration of letrozole +/- LHRH analogs should be continued as pre-planned schedule.

Patients discontinuing letrozole permanently due to treatment related toxicity will continue with abemaciclib in the active treatment phase of the study.

5.4.3. Doxorubicin, Cyclophosphamide and Paclitaxel or Docetaxel

Investigators should consult the locally approved prescribing information and investigator's criteria for all dose modifications/adjustments.

5.5. Medication Errors and Overdose

Medication errors may result in this study from the administration or consumption of the wrong drug, by the wrong patient, at the wrong time or at the wrong dosage strength. Such medication errors occurring to a study participant are to be captured on the adverse event

(AE) page of the eCRFs and on the SAE form when appropriate. In the event of medication dosing error, GEICAM should be notified immediately.

Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- ✓ Medication errors involving patient exposure to the product;
- ✓ Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating patient.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error and, if applicable, any associated adverse event(s) is captured on an adverse event (AE) eCRF page (refer to Management, Timing and Assessment of Adverse Events section for further details).

5.6. General Concomitant Medication and Supportive Care Guidelines

Patients must be instructed not to take any additional medication (over-the-counter or other products) during the study without prior consultation with the investigator. Any medications including herbal supplements, vitamins, or treatment taken by the patient from 28 days prior to the start of study treatment and up to 30 days following the last dose of study treatment and the reason for their administration must be recorded on the eCRF.

Routine postoperative care, such as dressing changes, suture removal, drain removal, or venous access (central or peripheral), does not need to be recorded. Anesthetics used for any surgical procedures performed during the patient's participation in the study can be recorded as "unspecified anesthesia" on the concomitant treatment records; it is not necessary to list the specific anesthetics. Palliative and supportive care for cancer-related symptoms will be offered to all patients in this study.

5.6.1. Prohibited Medications

The following treatments are prohibited throughout the duration of the active treatment phase:

- ✓ **Anticancer agents:** No additional investigational or commercial anticancer agents such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers, or endocrine therapy other than the ones stated in the protocol, will be permitted during the active treatment phase. In general, any drugs containing "for the treatment of breast cancer" on the product insert are not permitted on study.
- ✓ **Hormone replacement therapy**, topical estrogens (including any intra-vaginal preparations), megestrol acetate and selective estrogen-receptor modulators (eg, raloxifene) are prohibited during the active treatment phase.

- ✓ **Any concurrent radiotherapy:** is prohibited throughout the duration of the active treatment phase of the study. Patients requiring this procedure will be discontinued from the active treatment phase and will enter the follow-up phase. This radiotherapy will be considered an alternative cancer therapy and will result in censoring the patient.
- ✓ **Bisphosphonates and receptor activator of nuclear factor kappa B ligand** (RANKL), **used as anti-tumor treatment.**
- ✓ **In Arm A, luteinizing hormone-releasing hormone (LHRH) analogs** are not allowed regardless of the menopausal status.

5.6.2. Medications Not Recommended

The following treatments are not recommended throughout the duration of the active treatment phase. Alternative therapies should be considered whenever possible. If usage of the following treatments is deemed necessary, consultation and agreement with GEICAM is required prior to treatment initiation.

- ✓ **CYP3A inhibitors/inducers**: 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. Concomitant use of CYP3A inducers may reduce the activity of abemaciclib. Abemaciclib can be co-administered with drugs which are substrates of CYP enzymes.

  CYP3A inducers. At a minimum, it is recommended to avoid concomitant use of CYP3A inducers and consider alternative agents.

  CYP3A inhibitors. Concomitant use of strong and moderate CYP3A inhibitors may increase the likelihood of AEs related to abemaciclib. At a minimum, it is recommended to 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.

  Please, refer to **Protocol Attachment 5**. The information in this attachment is provided for guidance to investigators and does not preclude the use of these medications if clinically indicated.
- ✓ **Chronic immunosuppressive therapies** should be avoided, including systemic corticosteroids. Steroids given for physiological replacement, as anti-emetics or inhaled as well as short course of oral/topical steroids given for allergic reactions or asthma flares are allowed.

- ✓ The use of **herbal medicine** is not recommended during the active treatment phase.
- ✓ **Erythropoietin** should be avoided, taking into considerations the recommendations from ASCO/ASH Clinical Practice Guideline(65).

5.6.3. Permitted Medications

The following treatments are permitted throughout the duration of the active treatment phase:

- ✓ **Standard therapies** for pre-existing medical conditions, medical and/or surgical complications, and palliation. Any medication intended solely for supportive care (eg, analgesics, antidiarrheals, antidepressants) may also be used at the investigator's discretion. All medications should be recorded.
- ✓ **Hematopoietic growth factors** (eg, granulocyte colony stimulating factor [G-CSF], granulocyte macrophage colony stimulating factor [GM-CSF]): Primary prophylactic use of granulocyte-colony stimulating factors is not permitted but they may be used to treat treatment-emergent neutropenia as indicated by the current American Society of Clinical Oncology (ASCO) guideline. If neutropenic complications are observed in a cycle in which primary prophylaxis with CSFs was not received, secondary prophylaxis may be given at the discretion of the investigator, but only if dose reduction or delay are not considered to be a reasonable alternative.
- ✓ **Bone-sparing agents (e.g., bisphosphonates, denosumab for the treatment of osteoporosis/osteopenia)** are allowed in the study provided patients are on stable doses for at least 4 weeks prior to randomization.
- ✓ **Influenza and/or COVID-19 vaccines** should be preferably administered at the time of the cycle with the lowest myelosuppression.
- ✓ Caution is advised on theoretical grounds for any **surgical procedures** during the study:
    - o For **minor surgeries and procedures** (for example, ambulatory), investigators should treat as clinically indicated and closely monitor any signs of infection or healing complications.
    - o For **major surgeries**, the recommendation is to suspend dosing of abemaciclib for at least 7 days before and may be resumed as clinically indicated.
      Consider monitoring neutrophils and platelets before surgery and before resuming abemaciclib.
      The scars should be aseptic and healing process be reasonable before resuming abemaciclib.

Dose suspensions ≥ 28 days must be discussed with GEICAM.

5.6.4. General Guidance for Women of Child Bearing Potential and/or Use of Contraceptive Methods

Based on findings in animals, abemaciclib can cause fetal harm when administered to a pregnant woman. 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. Pregnant women should be advised of the potential risk to a fetus. Additionally, there are no available human data informing the drug-associated risk. A nursing woman should be advised to discontinue breastfeeding during treatment with abemaciclib.

- ✓ A female of childbearing potential, must have a negative serum pregnancy test within 7 days of the first dose of abemaciclib or CT and agree to use a highly effective contraception method during the treatment period and for 3 weeks following the last dose of abemaciclib or CT.
- ✓ 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 or CT should be reported. If a patient is determined to be pregnant following abemaciclib initiation, she must discontinue treatment immediately. Data on fetal outcome and breast-feeding are to be collected for regulatory reporting and drug safety evaluation.

5.7. Treatment Compliance

Patients will be required to return all bottles/blisters of abemaciclib and letrozole as well as the completed patient diary at each visit. Drug accountability will be performed prior to dispensing drug supply. The number of remaining tablets of abemaciclib, and letrozole will be documented and recorded.

The patient number should be recorded on the bottle/blister label at time of assignment to a patient. Site personnel must ensure that patients clearly understand the directions for self-medication. Patients should be given a sufficient supply to last until their next study visit. Unused drug and/or empty bottles/blister should be returned to the site at the next visit.

Patients included in the control arm B will be given a patient diary and will be asked to return the completed diary at each study visit in order to assess compliance.

To be considered compliant, each study patient must have received at least 80% of the planned number of doses of primary therapy based on the number of days of actual dose administration. Dose adjustments must follow instructions provided in the dose adjustment guidelines section.

Doxorubicin, cyclophosphamide and paclitaxel or docetaxel will be administered at the hospital and will be supervised by the investigator or his designee.

6. Efficacy and Safety Evaluations, Sample Collection and Testing (Standard Laboratory Testing) and Appropriateness of Assessments

Study procedures and their timing (including tolerance limits for timing) are summarized in the **Study Schedule**, **Protocol Attachment 1**.

6.1. Efficacy Assessments

All assessments to be performed at baseline and during the study are specified in the **Study Schedule**, **Protocol Attachment 1**.

6.1.1. Primary Efficacy Assessments

The primary efficacy variable is Residual Cancer Burden (RCB) 0-I rate.

RCB is a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden. It is estimated from routine pathological sections of the primary breast tumor site and the regional lymph nodes after the completion of NA therapy. The pathological variables include bidimensional diameters of the primary tumor bed, the proportion of primary tumor area containing invasive carcinoma, the number of positive lymph nodes, and the diameter of the largest nodal metastasis.

6.1.2. Secondary Efficacy Assessments

Secondary efficacy assessments are:

- Percentage of decrease in the geometric mean of Ki67 index value after 2 weeks of treatment in both treatments arms.

  Number of patients with cell cycle arrest (Ki67 < 2.7%) after 2 weeks of treatment in both treatment arms.

- RCB(23) is classified in four classes based on the residual disease (RD):
  - RCB-0 defined as pathological complete response.
  - RCB-I defined as minimal RD.
  - RCB-II defined as moderate RD.
  - RCB-III defined as extensive RD.
- Variation of the value of RCB based on the RD between the two treatment arms.
- PEPI(24) (Preoperative Endocrine Prognostic Index) requires pathological stage (tumor size and nodal status), level of Ki67 protein, and Allred ER score measured

on the surgical specimen. PEPI score 0 includes pT1 or pT2, pN0, Ki67 ≤ 2.7%, Allred score > 2.

Patients with a PEPI score of 0 (pT1 or pT2, pN0, Ki67 ≤ 2.7%, Allred score > 2) were found to have a low risk of recurrence (P024 recurrence(44, 66) and IMPACT(45, 67, 68) studies). In addition, recent results from ACOSOG Z1031 trial showed that recurrence risk over 5 years for patients with PEPI = 0 disease was 3.6% (versus 14.4% for patients with PEPI > 0).

Preoperative Endocrine Prognostic Index (PEPI)*				
Pathology, biomarker status	**Recurrence-Free Survival**		**Breast Cancer-Specific Survival**	
	HR	**Points**	**HR**	**Points**
Pathological tumor size				
T1/2	-	0	-	0
T3/4	2.8	3	4.4	3
Nodal status				
Negative	-	0	-	0
Positive	3.2	3	3.9	3
Ki67 level				
0% - 2.7% (0-1**)	-	0	-	0
> 2.7% - 7.3% (1-2**)	1.3	1	1.4	1
> 7.3% - 19.7% (2-3**)	1.7	1	2.0	2
> 19.7% - 53.1% (3-4**)	2.2	2	2.7	3
> 53.1% (> 4)	2.9	3	3.8	3
ER status, Allred score				
0-2	2.8	3	7.0	3
3-8	-	0	-	0

* The total PEPI score assigned to each patient is the sum of the risk points derived from the pT stage, pN stage, Ki67 level, and ER status of the surgical specimen. The total risk point score for each patient is the sum of all the risk points accumulated from the four factors in the model.

*The natural logarithm interval corresponding to the percent Ki67 values on the original percentage scale.

- Clinical Response Rate (CRR) is defined as the proportion of subjects with complete or partial radiographic response. CR and PR definitions are assessed by MRI at baseline and prior to breast surgery, with or without regional lymph nodes surgery, and categorized according to percent reduction in tumor size.

- Breast-conserving surgery (BCS) rate: defined as the proportion of patients who achieved breast conserving surgery between both treatment arms.
- Invasive event free survival (iEFS): defined as time from randomization to progressive disease or invasive disease recurrence (local, regional, distant, or contralateral), or death from any cause. Invasive disease recurrence is defined as:
    - Ipsilateral invasive breast tumor recurrence (including second primary invasive breast cancer): an invasive breast cancer involving the same breast parenchyma as the original primary lesion.
    - Ipsilateral regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, other regional lymph nodes, chest wall, and/or skin of the ipsilateral breast).
    - Distant recurrence (i.e., evidence of breast cancer in any anatomic site outside local and/or regional location and that has been either histologically confirmed or clinically diagnosed as recurrent invasive breast cancer).
    - Contralateral invasive breast cancer.
    - Second primary invasive cancer of non-breast origin.
- Safety will be assessed by standard clinical and laboratory tests (haematology, serum chemistry). AEs grade will be defined by the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) version 5.0. AEs terms will be coded according to MedDRA dictionary.
- Gene expression data provided by a multigene expression panel in sequential tumor biopsies.

6.2. Safety Assessments

Investigators are responsible for monitoring the safety of patients who have been enrolled in this study and for alerting GEICAM of any event that seems unusual.

The investigator is responsible for appropriate medical care of patients during the study.

The investigator remains responsible for following, through an appropriate health-care option, adverse events that are serious or that caused the patient to discontinue before completing the study. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator.

During the course of the study, all patients enrolled in the trial must be evaluated according to the schedule outlined in the flow charts and described below. The results of the evaluation will be recorded in the eCRF pages until the patients are not followed anymore.

Serious Adverse Event Reporting:

- To comply with applicable laws, regulations and standards regarding Investigator's and Institution's obligations, as the sponsor of the Study, to collect and report adverse events to regulatory authorities, IRBs, Ethics Committees or other third parties. In addition to the obligations set forth below, Investigator and Institution agree to provide Lilly with a copy of all information Investigator and/or Institution submit to regulators related to any adverse events for the Study Drug that occur during the Study that Investigator and/or Institution have not otherwise provided Lilly;

- to notify Lilly, sub-investigators, and the IRB of any problems involving risk to Study patients and report new safety information to IRBs in accordance with applicable requirements;

- to notify Lilly within fifteen (15) business days of Investigator and/or Institution receiving notification of any "serious" adverse event experienced by a patient participating in the Study and receiving Study Drug. For purposes of this requirement, "serious" means: (1) death; (2) in-patient hospitalization or prolonged hospitalization; (3) life-threatening; (4) persistent or significant disability or incapacity; (5) congenital anomaly or birth defect; or (6) other serious events that may jeopardize the patient and may require medical or surgical intervention to prevent one of the other five listed outcomes. Serious adverse events should be reported to Lilly using a CIOMS Form or other form acceptable to Lilly. 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.

6.2.1. Timing of Assessments

All assessments to be performed at baseline and during the study are specified in the Study Schedule, Protocol Attachment 1.

Vital signs assessments will include blood pressure, pulse and body temperature. Baseline standard FEVI is mandatory; and after treatment with antracyclines (only for Arm A), on the other visits it will be only performed if clinically indicated.

The following safety laboratory assessments will be performed by the local laboratories, at the times specified in the Study Schedule:

- Hematology*: hemoglobin, WBC, absolute neutrophils, lymphocytes, platelet count.

  Blood Chemistry*: fasting glucose, alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine, sodium, potassium, total calcium, urea (or BUN).

If the patient receives weekly paclitaxel 80 mg/m^2 for 12 weeks, hematology and blood chemistry can be performed every 21 days (each 3 week paclitaxel administration corresponds to one cycle).

* In arm B the haematology and blood chemistry will be performed every two weeks for the first two visits and from visit 3 onwards, every four weeks (± 3 days).

All AEs (and their relatedness to the study treatment) occurring during the study will be documented in the eCRF. AEs will be graded according to NCI-CTCAE version 5.0.

6.2.2. Definitions

The safety definitions are described in the **Table 9**.

Table 9: Safety definitions

Concept	Definition
Adverse Event (AE)	Any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product. Laboratory and vital signs abnormalities should be reported as AE only in case they lead to an action on study treatment or if they are serious. Any temporary increase in the severity of a symptom or previous sickness that happens after the baseline of the study is considered also as an AE.
Adverse Reaction (AR)	All untoward and unintended responses to a medicinal product related to any dose administered. All expected ARs are listed in the Investigator's Brochure (IB) in case of not authorized investigational product or Summary of Product Characteristics [SmPC] in case of an authorized investigational product. If the nature or the severity of an adverse reaction is not consistent with the

	applicable product information, the AR is defined as unexpected. The basis for the decision is the current version of the corresponding reference document that has been submitted and approved by the competent authority and the ethics committees. **Accountability criteria** The Sponsor will classify the adverse event, based in their causation relation with the investigational product, following the Karch y Lasagna (1977) algorithm, as: o Related (final, probable and possible): there is reasonable temporal sequence between the study treatment administration and the appearance of the adverse event. This event matches with the adverse reaction described for the investigational product, improves with the omission and reappears after its re-administration and can't be explained by other causes. o Not related (conditional or improbable or not related): there is no reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event does not matches with the adverse reaction described for the study treatment and can be explained by other causes. For expedited reporting purposes it is considered as related the categories: final, probable and possible from Karch y Lasagna (1977) algorithm and as not related the category conditional or improbable of that algorithm. The determination of the possible relation with the study treatment is responsibility of the principal investigator of the site or the person designated by him.
Serious Adverse Event (SAE) and Serious Adverse Reaction (SAR)	Any adverse event or adverse reaction that, at any dose: o is fatal (results in death), o initial or prolonged inpatient hospitalization, o a life-threatening experience (that is, immediate risk of dying, defined as an event in which the patient was at risk of death at the time of the event; it does not

	refer to an event which hypothetically might have caused death if it were more severe), o persistent or significant disability/incapacity, o congenital anomaly/birth defect or o an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (eg. medical, surgical) to prevent one of the other serious outcomes listed above. Do not confuse the concept "serious", described before, with "severe" which refers to the intensity of the AE or AR (minor/mild/severe). The following events will be considered as Special situations events and they have to be documented as a SAE and notified to the Pharmacovigilance Department of GEICAM immediately: overdose, misuse and abuse inadvertent or accidental exposure to investigational product, medication error, suspected transmission of an infectious agent via IP and cancer (except for breast cancer).
Suspected Unexpected Serious Adverse Reaction (SUSAR)	Any serious adverse reaction whose nature, intensity or consequences do not correspond with the reference information for the investigational product (example, Investigator Brochure [IB] in case of not authorized investigational product or Summary of Product Characteristics [SmPC] in case of an authorized investigational product). The unexpected nature of an adverse reaction is based in the fact of not being observed previously and not in what could be advanced based on the pharmacological properties of the study treatment.

6.2.3 Management, Timing and Assessment of Adverse Events

AE Classification	Adverse events should be classified following version 5.0 of the Common Terminology Criteria for Adverse Events

	(CTCAE) of the National Cancer Institute (NCI). A copy can be downloaded in the NCI web site: http://evs.nci.nih.gov/ftp1/CTCAE. The investigators team must have access to the CTCAE-NCI version 5.0. The AE not included in the CTCAE will be classified as described on Protocol Attachment 3. The causal relation between the investigational product and the AE will be assessed by the investigator using the Karch y Lasagna (1977) algorithm.
Procedure to notify an AE to GEICAM	The site must notify to GEICAM, through eCRF, the following events: o All adverse events that occur after signed Informed Consent Form. o Preexisting conditions that get worse during the study. o The evaluation of the possible relationship of each adverse event to the study treatment or protocol procedure. o The circumstances and data that causes the suspension of the treatment of a patient due to an adverse event. o The events related with progression/recurrence will not be recorded as adverse events, unless the investigator believes they could have been caused by the study treatment. o The events leading to the clinical outcome of death from disease progression will not be recorded as adverse events, unless the investigator believes they could have been caused by the study treatment.
Timing and assessment of AE/SAE (see Protocol Attachment 4)	The site staff will report on the eCRF the information of the AE/SAE in the following periods: • **Baseline (after the patient signs the ICD and before study treatment administration):** study site personnel will note the occurrence and nature of each

	patient´s medical condition(s) and preexisting conditions in the appropriate section of the eCRF. If a patient never receives study treatment but experiences an AE/SAE after the ICD is signed, ONLY events the investigator believes may have been caused by a protocol procedure will be reported to GEICAM via eCRF and SAE form (if applicable). • **During treatment with the study treatment:** during the study treatment administration, site personnel will record any change in the condition(s) and the occurrence and nature of any AE/SAE. A CTCAE grade rating will be assigned before each cycle for any AE experienced during the previous cycle. • **30-day** (±7 days) **post-treatment follow-up period:** for safety reasons all patients will have a visit after finishing treatment with the study medications. In **Arm A** patients will have this visit 30 (±7) days after finishing treatment with the study medications. In **Arm B** the visit will be done within 7 days from the last abemaciclib and/or letrozole dose. Patients should be closely followed for study treatment AE/SAE in order to detect delayed toxicity. If study treatment-related toxicity is present beyond 30 days post-treatment, patients must be followed until it resolves or improved to baseline, the relationship is reassessed as unrelated, the investigator confirms that no further improvement can be expected, another therapy is initiated, or death. • **Long-Term Follow-up Period (after the 30-day (±7 days) post-discontinuation/post-treatment):** ongoing SAEs, and new SAEs thought to be related to study treatment or protocol procedures should be documented on the eCRF and immediately reported to GEICAM via the designated transmission method. If the study has been closed the notification to GEICAM will be done via the designated transmission method.

	• **SAES related with study treatment** should be collected and analyzed until they are solved or until the toxicity is considered irreversible.
Reporting of Study Specific AE/ Adverse Events of Special Interest (AESIs)	Adverse Events of Special Interest (AESIs) for abemaciclib are: diarrhea, neutropenia, liver enzyme increased, venous thromboembolism (VTE) and infections. AESIs must be reported in eCRF. If an AESI meets seriousness criteria, it must be reported as a SAE according to the procedure described in section 6.2.4.

6.2.4. Management, Timing and Assessment of SAEs/pregnancies

Timing of SAEs (see Protocol Attachment 4)	All the SAEs (either spontaneously or during the trial visits) will be collected since the patient signs the Informed Consent Document (ICD) see Attachment 4. All the SAEs must be documented in the medical record of the patient and in the eCRF. A follow up of all the SAEs should be done until they are solved or until the toxicity is considered irreversible.
Pregnancies	If a patient becomes pregnant while enrolled on a GEICAM study, it must be reported in the Pregnancy Form and sent to the GEICAM pharmacovigilance department within 24 hours of becoming aware of it.
SAEs which do not need to be notified to the Pharmacovigilance Department of GEICAM	The following events are not considered SAEs: o A visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event). o Elective surgery planned before signing consent. o Hospitalization which is due solely to a planned study visit and without prolongation. o Routine health assessment requiring admission for baseline/trending of health status (eg. routine colonoscopy). o Medical/surgical admission for purpose other than remedying ill health state that was planned before

	study entry. Appropriate documentation is required in these cases. o Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg. lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative). o Progression of the malignancy during study (including signs and symptoms of progression), unless the outcome is fatal and death occurred before the end of treatment. Thereafter death due to disease progression. has not to be reported as SAE. They will be reported in the eCRF and in the patient record. o Hospitalization due to signs and symptoms of disease progression. o An overnight stay in the hospital that is only due to transportation, organization or accommodation problems and without medical background. The rest of SAEs must be notified as described as follows.
Procedure to notify a SAE to the Pharmacovigilance Department of GEICAM	The SAE reporting to GEICAM is required to comply with applicable laws, regulations and standards regarding Investigator's and Institution's obligations, as the sponsor of the study, to collect and report AEs to Regulatory Authorities, IRBs, Ethics Committees or other third parties. The SAEs must be notified to the Pharmacovigilance Department of GEICAM. A member of the investigator team must complete and sign the GEICAM SAE notification form which will be sent by fax/mail, immediately and always during the 24 hours following knowledge of the SAE. **Pharmacovigilance Department of GEICAM** Fax: [redacted] [redacted] GEICAM will review the received form and, if necessary, will ask more information to the investigator.

	When additional information is obtained about the SAEs, or this is solved or is improbable it will change, a follow-up report must be also completed and sent by fax/mail, immediately and always during the following the 24 hours to the Pharmacovigilance Department of GEICAM. If GEICAM suspects that the SAE could be a SUSAR, the investigator should give the follow up information requested. All SAEs/AESIs from the time the patient have the first dose of the study treatment through 30 days following the last administration of study reatment must be reported according to the procedure described below. All SAE regardless of timing must be reported, if considered related to study treatment. Likewise, progression of a patient's underlying condition leading to one of the above should also not be reported as a SAE. GEICAM will report all SAEs and AESIs immediately to the Chief Investigators. All SAEs and AESIs will be followed-up by the investigator until satisfactory resolution. Annually all SARs will be reported at the DSUR to the competent authorities and the leading ethics committee, including all SUSARs. Withdrawal from further treatment shall be at the discretion of the investigator.
Overdose	A non-relevant overdose (accidental or intentional) with the study drug is an event suspected by the investigator or spontaneously notified by the patient and defined as: ✓ The intake up to 8 tablets of abemaciclib in the same day. ✓ The intake of 180 tablets in a 28-day cycle. It is to be reported to GEICAM in the eCRF.

	A relevant overdose (accidental or intentional) with the study treatment is an event suspected by the investigator or spontaneously notified by the patient and defined as: the administration of a quantity of a medical product which is above the maximum recommended dose according to the product information. It has to be reported within one working day as a SAE on a SAE form.
Death on Study	Any death occurring during the active treatment part of the study and within 30 days following the last treatment must be reported to GEICAM as the Sponsor within 24 hours, regardless of the relation to study treatment, and has to be reported on the death report form section of the eCRF. The cause of death should be documented (cancer-related, treatment-related, cancer- and treatment-unrelated). Autopsy reports should be collected whenever possible and sent to the GEICAM. Deaths that occur due to tumor progression do not have to be reported as a SAE unless they occurred before end of treatment. Deaths after the end of study which are considered to be related to study treatment have to be reported as SAEs. To the extent feasible sufficient information including relevant laboratory values, ECG, scan, biopsy or autopsy results must be provided by the investigator in the SAE narrative (even if investigator determines the SAE is not related) so as to permit an independent causality assessment by a Competent Authority.

6.2.5. Management, Timing and Assessment of SUSARs

Expedited Notification of SUSAR to the Competent Authorities and EC/IRB	The Pharmacovigilance Department of GEICAM or its designee is responsible to notify to each of the competent authorities and EC/IRBs of the participating countries, all the SUSARs collected in the study, following the procedures shown in the current legislation.

Timing of notification	The deadline for reporting a SUSAR shall be 15 calendar days from when GEICAM or its designee becomes aware of it. When suspected SUSAR caused the death of the patient or endangered her life, GEICAM or its designee will send the information within 7 calendar days from the date on which it becomes aware.
Expedited reporting of other relevant safety information	GEICAM or its designee will also notify, expeditiously, all the information that could modify the balance benefit/risk of the investigational product, or determine changes in its administration pattern or in the study performance, such as: o A qualitative change or an increase in the percentage of occurrence of the SAR expected, which are considered clinically significant. o The SUSAR occurring after completion of the study and are reported by the investigator to the Sponsor. o New events related to the conduct of a trial or the development of an IMP likely to affect the safety of patients, such as: ✓ SAE that could be related with the study procedure and could modify the conduct of the trial. ✓ A significant risk to patients such as lack of efficacy in a drug used to treat a life-threatening illness. ✓ A major safety finding from a newly completed animal study (such as carcinogenicity). ✓ A temporary halt of a trial for safety reasons if the trial is conducted with the same investigational medicinal products in another country and if this information is known by GEICAM. This relevant information shall be notified as soon as possible and no later than 15 days after GEICAM or its designee becomes aware of it. Additional information will also be notified as quickly as possible.
Development Safety	The DSUR that include the SAEs and SUSARs collected

Update Report (DSUR)	during the study will be sent by GEICAM or its designee to the Competent Authorities and EC/IRB at the time established by the current legislation.
Notification to investigators	GEICAM or its designee will communicate to the investigators any safety information that may affect the safety of trial patients, as soon as possible. Information on SUSAR occurred during the study will be sent to investigators every 6 months, in aggregate, in a list along with a brief analysis of the data provided. They will be informed also, throughout the entire study, of any safety aspect that impacts the performance on the clinical trial or in the product development, including the interruption or modification in the development program of the protocol safety-related.

6.3. Other Assessments

6.3.1. Biomarker Assessments

Detailed instructions for the collection, handling and shipment of samples are outlined in the Sample Management Manual (SMM) that will be available to the investigator and will be distributed at the time of site activation.

The following samples will be collected and analyzed for molecular characterization (for example, analysis of pathways activation, gene expression profiling, evaluation of immune response, etc):

1. **Tumor Tissue:**

   1.1. **For Ki67 screening:** pre-treatment formalin-fixed paraffin embedded (FFPE) core tumor biopsy (fine needle aspiration will not be allowed).

   In case of patients with 2 or more tumor lesions: if all histologically available lesions have similar morphological characteristics (i.e. based on local grade, type, Ki67 range…), only the tumor lesion with bigger size will be sent to central laboratory for Ki67 assessment. If tumor lesions have different morphological characteristics, all discordant tumor lesions will be centrally evaluated for Ki67.

   For eligibility into the study, tissue of breast tumor samples collected prior to exposure to any systemic therapy from potential patients must be sent to the

designated central laboratory, where Ki67 status will be evaluated (as described in Section 4.1, Inclusion Criteria) using a Ki-67 IHC assay.

The use of Ki-67 IHC assay to identify patients for eligibility into the study is supported by a risk assessment by Sponsor. Clinicians will consider each patient's disease, other eligibility criteria, and the Ki-67 IHCassay result, to decide on study eligibility. No treatment decision shall be made solely on the basis of the Ki-67 IHC assay result. However, in order to be eligible to be enrolled, a patient must have a Ki-67 IHC assay diagnostic-positive status with Ki-67 labeling index of ≥ 20%.

Agilent's Ki-67 kit is a standardized IHC assay that detects expression of Ki67 in FFPE BC tissue specimens using a Ki-67 antibody that is CE-marked for detection of Ki67 antigen in normal and neoplastic cells. The investigational Ki-67 IHC kit is manufactured by Agilent Technologies, Inc. This IHC assay is a validated assay (the kit protocol and components are locked, manufactured in a GMP facility and analytically validated) and comply with regional regulatory requirements for investigational devices. For the purposes of this study, the kits used will be labeled "for performance evaluation only".

1.2. For secondary and exploratory biomaker analysis:

1.2.1. Pre-treatment sample:

According to the Sample Management Manual, FFPE tumor tissue will be provided from clinical sites to perform the analysis of a multigene expression platform (OncotypeDx®), in order to explore the downstaging of risk category [based on the Recurrence Score (RS)]. Additionally, one FFPE core biopsy will be required for biomarker analysis if there is not enough tumor tissue available after screening.

In selected sites, an additional core needle fresh tumor biopsy must be collected for generation of organoid-based models and/or xenographs.

1.2.2. After 2 weeks of treatment (between day 14 and day 21, preferably as close to day 14 as possible): FFPE core needle biopsy will be required (fine needle aspiration will not be allowed). The lesions had to be centrally evaluated at baseline.

1.2.3. Tumor surgery specimen: FFPE tumor block. The lesions had to be centrally evaluated at baseline.

Additionally, in selected sites, fresh tumor tissue post-treatment will be collected for generation of organoid-based models and/or xenographs (fine needle aspiration will not be allowed).

To comply with secondary end points of the study, we will evaluate Ki67 at 2 weeks to evaluate potential decrease of Ki67 after treatment, and Ki67 level at surgery for assessment of endpoints as PEPI score. Additionally, downstaging of risk category based on multigene expression platforms will be assessed (a multigene expression platform used for risk classification, could be OncotypeDx®, based on the RS; GEICAM could provide the investigators with the RS results from pre-treatment samples of patients once the breast surgery has been performed, so this information could be considered by the investigators to plan the adjuvant treatment for these patients). No patient management decisions are made based on the Ki67 results related to these secondary endpoints.

Other tumor tissue biomarkers, including DNA, RNA and protein analytes could be analyzed to investigate possible associations with resistance/sensitivity to treatment. Biomarkers that will be analyzed will be selected based on their known relevance to mechanisms involved in breast cancer development and therapy related pathways. Examples of such biomarkers could be *TP53* and *MYC* mutations, *MYC* copy number variations (CNVs) or tissue expression of CD4/CD8/FOXP3/PDL-1. The relationship between centrally assessed biomarkers and the outcome or the resistance/sensitivity to treatment will be reported in an exploratory fashion. The prognostic or predictive relevance of intrinsic subtypes, genomic profiles and/or molecular patterns could be evaluated using genomic, transcriptomic, proteomic and metabolomic analysis. Highthrough-put techniques, for example, whole genome/exome next-generation sequencing (NGS) and whole transcriptome RNA sequencing (RNA-Seq) could be performed on pre-treatment and surgery tumors to explore the genetic changes in tumors after NA therapy.

The generation and expansion of ex vivo organoid-based models and/or xenographs from post-therapy tumors and from baseline tumors will be pursued to carry on high-throughput functional screening studies.

2. **Blood Biospecimens**:

2.1 Plasma and whole blood samples for ctDNA exploratory biomarker analysis will be collected at the following timepoints:

- **Treatment phase**: at baseline (within 7 days prior to initiate study treatment), at 2-3 weeks (wk), at 12 wk (+/- 1 wk), and at EOT for any reason (collected within the following 2 wk, prior to surgery) and at 4 weeks post-surgery (+/- 2 wk, collected before starting adjuvant CT).

- **Follow-up phase**: every 6 months (+/-28 days), during the first five years, every year (+/-28 days) during the following 5-years and after recurrence, whatever occurs first.

2.2. Plasma and/or whole blood samples for other biomarker analysis (e.g. metabolic and immune response analysis): pre-treatment (within 7 days prior to initiate study treatment), 2-3 wk, 12-wk (+/- 1 wk), 6 months (+/- 2 wk) for both arms (EOT for arm A, if it occurs before 6 months), and EOT for arm B before surgery. A local serology report performed at any time along the study, but preferably at baseline visit, should be sent to the central laboratory. This serology must include at least HIV, hepatitis B and hepatitis C and, only if available, also hepatitis A, Human Papillomavirus (HPV) and Pox virus.

Plasma and/or whole blood samples will be retained for potential biomarker analyses related to treatment response and treatment metabolism. Biomarker exploratory analysis would include at least characterization of mutational genetic profiles and monitoring residual disease and tumor burden in ctDNA samples along the study, as well as proteomics, metabolomics and immune response related analyses. Genomic, epigenetic, proteomic and metabolomic variation may help to explain some of the variability in response seen with some drugs among different individuals. Comparing the DNA, RNA, protein, and metabolite patterns of patients who respond well and those who respond poorly to treatment may help to better define the most appropriate group of patients in which to target a given treatment. Collecting samples for biomarker analyses and retaining them makes it possible to seek explanations for differences in, for example, exposure, efficacy, tolerability, or safety not anticipated prior to the beginning of the study.

All biomarkers will be correlated with efficacy endpoints or surrogate endpoints, as feasible. The relationship between centrally assessed biomarkers and the outcome or the resistance/sensitivity to treatment will be reported in an exploratory fashion. Additional exploratory analysis might be performed and described in a previous Statistical Analysis Plan.

7. Data Quality Assurance

To ensure accurate, complete and reliable data, GEICAM will do the following:

- Provide instructional material to the study sites, as appropriate.
- Sponsor a start-up training session to instruct the investigators and study coordinators. This session will give instructions on the protocol, the completion of the eCRFs, and study procedures.
- Make periodic visits to the study site to review study progress, investigator and patient compliance with the clinical trial protocol requirements and any emergent problems.
- Be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax.
- Review and evaluate eCRF data and use standard computer edits to detect errors in data collection.
- Conduct a quality review of the database.
- Verify the quality of the data.

To ensure the safety of participants in the study and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as original source documents for the study. If requested, the investigator will provide GEICAM, applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents.

7.1. Data Management and Registries file

Data for this study will be recorded in an eCRF with web access, designed, created and maintained by GEICAM using Oracle Clinical®. Data will be transcribed by the site staff from the source documents onto the eCRF. The eCRF will never be considered as source data for this trial.

This eCRF meets the 21 CFR part 11, ensuring the validation of the system, the traceability and the audit trail, the retention, protection, reproducibility and recovery of trial data, control of access to information and electronic signature, among others.

Visit data should be entered into the eCRFs within 5 business days. Each eCRF should be completed by the investigator or delegate as stated in the Site Delegation List.

Electronic queries will be raised if data is unclear or missing. GEICAM will perform a data review and additional requests can be sent through the eCRF, which the investigator is

obliged to respond to by modifying or clarifying the data questioned. The requests with their responses will be managed through the eCRF.

If a correction is made, the corrected information will be entered into the eCRF, overwriting the initial information. An audit trail allows you to identify the modification.

8. Sample Size and Statistical Methods

8.1. Determination of Sample Size

8.1.1. Sample Size determination

A Bayesian design has been used to define the most appropriate sample size:

The major advantage of this Bayesian approach is it allow us to evaluate how similar response rates between both treatment arms are, without using a very large non-inferiority study.

Comparability of RCB0/1 will be declared if the following Bayesian criterion is achieved in the primary RCB0/1 analysis:

$$\text{Posterior } P(\text{true RCB0/1}_{\text{Chemo}} - \text{true RCB0/1}_{\text{Abema+AI}} < 5\%) > 80\%$$

Full detail regarding the Bayesian model for ORR (including prior specifications) will be provided in the statistical analysis plan (SAP).

Simulations were conducted to evaluate probability of declaring comparability based on various scenarios of underlying true RCB0/1 values. Different underlying true RCB0/1 values for abemaciclib plus an AI were considered in the scenarios.

Three scenarios were explored (n=150, n=200, and n=250) and it is considered that the 2nd scenario with a sample size of 200 patients is the best option. Simulation results are shown in the table below.

$RCB0/1_{Chemo}$	$RCB0/1_{Abema+AI}$	N=150	N=200	N=250
0.16	0.16	57%	60%	61%
0.16	0.20	77%	82%	86%
0.16	0.24	90%	94%	96%
0.16	0.28	96%	98%	99%

8.2. Statistical and Analytical Plans

8.2.1. General Considerations

Statistical analysis of this study will be the responsibility of GEICAM. The interpretation of study results will be the responsibility of the principal investigator of the study.

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated and maintained by GEICAM. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

All analysis will be performed using the SAS Enterprise Guide 7.1 version.

8.2.1.1. Patient Populations

Intent to treat population (ITT): the ITT population will include all patients who are enrolled according to the initial treatment assignment. The ITT population will be the primary population for the efficacy analysis. It will be performed a sensitivity analysis using the Per-protocol population.

Per-protocol population: a subset of the ITT population that received at least one dose of study treatment and completed the study without any major protocol deviations according to the protocol deviation manual.

Safety population: will include all patients enrolled in the study who received at least one dose of study treatment, according to the actual treatment received. This population is for the safety analysis.

Biomarker population: a subset of the safety population with available samples and clinical data for biomarker analyses.

8.2.2. Patient Disposition

A detailed description of patient disposition will be provided. It will include:

- summary of patients screened and by site
- total number of patients screened
- total number of patients enrolled
- total number of patients treated
- summary of reasons for patients enrolled, but not treated.

A detailed summary of reasons for patient discontinuation from study treatment will be provided.

A summary of all identified important protocol violations will be provided.

8.2.3. Patient Characteristics

Patient characteristics will include a summary of the following:

- patient demographics
- baseline disease characteristics
- preexisting conditions/secondary conditions
- prior therapy

Other patient characteristics will be summarized as deemed appropriate.

Standard descriptive statistics, such as the mean, median, range and proportion, will be used to summarize the patient sample and to estimate parameters of interest. Ninety-five percent confidence intervals will be provided for estimates of interest where possible.

8.2.4. Concomitant Therapy

A summary of concomitant therapies will be generated in the safety population.

8.2.5. Treatment Compliance

Treatment information will be collected at each dose administration. The estimate of percent compliance will be given by:

$$\text{Percent Compliance} = \frac{\text{Actual dose administered per week}}{\text{Dose expected to be administered per week}} \times 100$$

No minimal level of compliance will be defined for patient inclusion in efficacy analyses. To be considered compliant patients should have received at least 80% of the planned number of doses. Exploratory analysis of the impact of compliance on selected efficacy endpoints may be performed if deemed necessary. More information can be found in SAP.

8.2.6. Efficacy Analyses

All efficacy definitions are described in section 6.1.

All efficacy analysis will be based on the ITT population. Additional efficacy analyses will be performed on the PP population.

8.2.6.1. Analyses of Primary Endpoint

The primary endpoint is Residual Cancer Burden (RCB) 0-I rate in both treatment arms.

Residual Cancer Burden (RCB): The information about the result of RCB will be provided by the local laboratory. If the patient has no information about the RCB the patient will be considered as non-responders in the RCB rate analysis.

RCB rate on each treatment arm will be estimated by dividing the number of patients with RCB 0-I by the ITT patients with measurable disease by treatment arm (“response rate”).

$$\text{RCB Rate} = \text{Number of RCB 0-I}$$

ITT population with measurable disease

Comparability between the two treatment arms will be assessed using the following Bayesian criterion:

$$\text{Posterior } P(\text{true RCB0/1}_{\text{Chemo}} - \text{true RCB0/1}_{\text{Abema+AI}} < 5\%) > 80\%$$

Posterior medians and Bayesian credible intervals will be reported for the RCB0/1 rate for each arm. In addition, arms will be compared using a Cochran-Mantel-Haenszel (CMH) test using the stratification factors. The accompanying 2-sided 95% confidence intervals (CIs) in each arm and the difference between arms will be computed.

Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

8.2.6.2. Analysis of Secondary Endpoints

The secondary efficacy endpoints are:

Changes in Ki67 index value after 2 weeks of treatment in both treatment arms: it will be evaluated the Percentage of decrease in the geometric mean of Ki67 index value after 2 weeks of treatment in both treatments arms. Values after 2 weeks of treatment will be expressed as geometric mean proportion of the baseline and transformed into percentage changes. An ANOVA analyses will be conducted at a two-sided 5% significance level for a within-treatments and between-treatment comparison. This analysis will be conducted in the ITT population.

Also to measure the changes in Ki67 after 2 weeks of treatment it will be assessed the number of patients with cell cycle arrest (Ki67 < 2.7%) after 2 weeks of treatment in both treatment arms in the ITT population.

Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

Residual Cancer Burden (RCB): RCB is classified in four classes based on the residual disease. It will be assess the differences between RCB 0+I vs. RCB-II vs. RCB-III in both treatment arms, also the distribution of RCB on each arm will be estimated. For this analysis it will be used the ITT population.

Kruskal-Wallis test will be used to examine differences between treatment arms.

Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

Residual Cancer Burden (RCB) value: RCB value will be obtained from the residual disease. It will be evaluate the differences of mean between both treatment arms. For this analysis it will be used the ITT population.

The T-test or Wilcoxon test will be used to examine differences between treatment arms.

Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

Rate of PEPI score 0 at breast surgery: Rate of PEPI score of 0 at baseline will be estimated by dividing the number of patients with PEPI score of 0 at surgery by the ITT patients in both arms.

$$\text{Rate of PEPI score 0 at surgery} = \frac{\text{Number of patients with PEPI score 0 at surgery}}{\text{ITT patients}}$$

This rate will be reported, including a 95% confidence interval.

CRR rate comparison between the two treatment arms will be assessed using CMH test with the same stratification factors. Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

Clinical Response Rate (CRR): A patient will be considered to have achieved a CRR if the patient has a sustained complete response (CR) or partial response (PR) assessed by MRI. Otherwise, the patient will be considered as non-responders in the CRR rate analysis. Additionally, patients with inadequate data for tumor assessment (eg, no baseline assessment or no follow-up assessments) will be considered as non-responders in the CRR rate analysis.

CRR rate on each treatment arm will be estimated by dividing the number of patients with objective response (CR or PR) by the ITT patients with measurable disease by treatment arm ("response rate").

$$\text{Objective Response Rate} = \frac{\text{Number of CRs + PRs}}{\text{ITT population with measurable disease}}$$

The CRR rate will be reported, including a 95% confidence interval.

In addition, the best clinical response for each patient will be summarized by treatment arm. CRR rate comparison between the two treatment arms will be assessed using CMH test with the same stratification factors.

Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

Rate of breast conservative surgery (BCS): Defined as the proportion of patients who achieved breast-conserving surgery between both treatment arms.

BCS rate on each treatment arm will be estimated by dividing the number of patients with conservative surgery by the ITT population.

$$\text{BCS Rate} = \frac{\text{Number of patients with conservative surgery}}{\text{ITT patients}}$$

The BCS rate will be reported, including a 95% confidence interval.

BCS rate comparison between the two treatment arms will be assessed using CMH test.

Invasive Event Free Survival (iEFS): iEFS data will be censored on the date of the last tumor assessment on study for patients who do not have progression or invasive recurrence (local, regional, distant, or contralateral), and who have not died due to any cause while on study.

iEFS will be analyzed in the ITT population. A stratified and non-stratified log-rank test (two-sided) will be used to compare iEFS time between treatment arms at the final analysis. iEFS for the two arms will be assessed using Kaplan-Meier methods and displayed graphically where appropriate. The median event times and 95% CIs will be estimated. Cox regression models will be used to estimate the treatment hazard ratio and its 95% confidence interval. Additionally a similar analysis will be also performed in the PP population as a sensivity analysis.

8.2.7. Safety Analyses

The toxicity and tolerability of study treatments will be evaluated in the safety population. Safety analyses will include summaries of the incidence of adverse events by maximum CTCAE grade v5.0that occur during the study treatment period or within 30 days of the last dose of study treatment, regardless of causality and according to the relationship to study treatment as assessed by the investigator. Additionally, the following safety-related outcomes will be summarized:

- study treatment discontinuations due to adverse events,
- deaths,
- SAEs,

- hospitalizations and transfusions.

Analyses for data with discrete dates, for example, deaths, transfusions, and concomitant medications, will be done through 30 days after each patient's last dose of study treatment. Adverse events will also be analyzed in this timeframe. Serious adverse events not related with study treatment will be collected and analyzed from enrollment till 30 days after each patient´s last dose.

Adverse events data and serious adverse events will be presented in frequency tables by grade. Hematological and clinical biochemistry toxicities will be assessed from laboratory test parameters. The safety analysis will be performed in the safety population.

8.2.8. Other Analyses

8.2.8.2. Biomarker Analyses

The biomarker analysis of the present study will be exploratory and primarily make use of descriptive statistical methods. For continuous variables, descriptive statistics including the mean, standard deviation, median, minimum, and maximum values, will be provided (by treatment arm, if applicable). Categorical variables will be summarized by numbers and proportions, and provided by treatment arm. In all possible cases, the 95% confidence intervals will be calculated. If appropriate, a chi-squared test will be used to test group differences for categorical variables.

If applicable, parametric test (analysis of variance or t test) or non-parametric testing, such as Wilcoxon's rank-sum test or Kruskal–Wallis test for continuous variables, and the Pearson $\chi 2$-test or Fisher exact test for categorical variables will be used to test group differences. All test performed will be two-sided and carried out with a 5% α-error rate without correction for multiplicity.

Rate of RCB 0-I, PR, CR and/or CRR and other efficacy endpoints or surrogate endpoints will be reported, including a 95% confidence interval. Comparison of these endpoints between the two treatment arms will be assessed using CMH test or Kruskal-Wallis test. The Kaplan-Meier limit-product method could be used to estimate other efficacy endpoints (ej. iEFS). The comparison of those endpoints between groups would be performed using the Log-Rank test. The Kaplan-Meier survival curves will be presented graphically. Median (EFS, iEFS, etc.) with the 95% confidence interval will be reported. Cox regression models will be used to estimate unadjusted and adjusted hazard ratio and its 95% confidence interval. The Wald test will be used to establish the prognostic importance of each variable. Univariate and multivariate analyses could be carry out to explore the influence of the selected variables in rate of RCB 0-I, CRR, iEFS, etc. If it is appropriate, additional statistical analyses will be performed to investigate any possible relationship of

biomarker levels with outcome and/or chemotherapy or abemaciclib plus endocrine therapy anti-tumor efficacy. Any additional sensitivity analyses will be outlined in specific SAPs.

8.2.9. Subgroup Analyses

Exploratory subgroup analysis may be performed if deemed appropriate.

8.2.12. Criteria for End of Study

This study will be considered complete following the data cut-off date and datalock for the final analysis. The data cut-off date for the final analysis will occur after all enrolled patients have been followed for at least 10 years or withdraw of the informed consent, whatever occurs first.

If further data are collected that are not included as part of the final locked database, the postlock data will eventually be combined with the locked database and stored in a data library separate from the locked database.

The end date of study is the date of the last visit of the last patient including follow up.

Performing exploratory analysis will be independent of the date of the end of the study.

9. Informed Consent, Confidentiality, Responsibility Insurance and Regulatory Considerations

9.1. Informed Consent

The investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the study, including answering any questions the patient may have throughout the study and sharing any new information that may be relevant to the patient's willingness to continue his or her participation in the trial in a timely manner.

The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is screened into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study.

The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study treatment.

As used in this protocol, the term "informed consent" includes all consent and assent given by patients or their legal representatives.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

9.2. Respect of Confidentiality

The investigator will be responsible for preserving the suitable information about each patient (for example, name, address, telephone number, social security number and study identification) so that the competent authorities can have access to said information if necessary. These records must be confidentially preserved for the time indicated by the legislation.

The investigators and GEICAM and its designee will maintain the confidentiality of all patients participating in the study, according to Good Clinical Practice, GCP and local legislation.

This clinical trial will be held in accordance and in compliance with local current legislation. Any treatment of personal data that is held within the clinical trial, for Sponsor, Principal Investigator, Site, and / or any other participant in the clinical trial, especially as far as informed consent, shall conform to the provisions of General Data Protection

Regulation (EU) 2016/679 with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC and any other applicable normative.

9.3. Insurance and Compensation for Injury

The Sponsor shall ensure that the trial subjects are compensated for any damage suffered as a result of the trial.

The trial Sponsor has hired an insurance policy covering the damage, as well as any liability that might be incurred by the Sponsor, principal investigator and members of the investigator team, including contracted clinical investigators, and the hospital or site where the clinical trial is conducted, according to applicable legislation.

9.4. Regulatory Considerations

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices and the applicable laws and regulations. Each investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator". The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

9.4.1. Investigator Information

Physicians with a specialty in medical oncology will participate as investigators in this clinical trial.

If investigators are added after the study has been approved by GEICAM, an EC/IRB, or a regulatory agency, these additions will not be considered changes to the protocol.

9.4.2. Protocol Signatures

After reading the protocol, each principal investigator will sign the protocol signature page and send a copy of the signed page to GEICAM.

10. Practical Considerations

This trial will be managed using a risk-based approach. Critical processes/ data identification and risk identification, evaluation, control, communication and review will be performed according to GEICAM SOPs and will be incorporated to the Risk Management Plan of the study.

10.1. Monitoring, Audit and Inspections

Onsite and/or remote monitoring visits to the study site will be made periodically during the study and according to the Monitoring Plan Document to ensure that all aspects of the protocol are followed. During the visits to the site, the monitor must review the original records of the patients, the records of medication stocks and document preservation according to the Monitoring Plan Document. The monitor must evaluate the study procedures and discuss the possible problems with the investigator. During the course of the study, audit visits can be carried out in the participating sites. The investigator will allow direct access to the source documents/data for the tasks of monitoring, audit, reviewed by the EC/IRB and the inspection by the Competent Authorities.

10.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study subjects. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the investigator should consult with GEICAM or designee (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospectively approved deviation) from the inclusion or exclusion criteria.

10.3. Preservation of Study Documentation

The copies of all the relevant information will be preserved by the investigator for a period of at least 25 years after the end of the study, according to current legislation.

10.4. Protocol Modification

Once it has been authorized by the EC/IRB and the competent authority any protocol modification must be documented by writing, in the form of an amendment.

The amendments must be duly identified, by its chronological order number, dated and signed by GEICAM and the Chief investigator.

The protocol amendments considered as substantial must be notified to the EC/IRBs involved in the trial and to the competent authority. The authorization of the involved EC/IRBs and/or the competent authority will be necessary before their application.

After reading the protocol amendment, each principal investigator will sign the protocol amendment signature page and send a copy of the signed page to GEICAM.

Any change in the study plan requires a protocol amendment. An Investigator must not make any changes to the study without EC/IRB and Sponsor approval except when necessary to eliminate apparent immediate hazards to the patients. A protocol change intended to eliminate an apparent immediate hazard to patients may be implemented immediately, but the change must then be documented in an amendment. All protocol amendments must be reviewed and approved, by the Sponsor.

10.5. Use of the Information and Publication

All the information concerning the study treatment provided by GEICAM in relation to this study, and not previously published, is considered to be confidential information with property right of GEICAM. This information comprises the basic information about the product, the clinical protocol, the work forms where appropriate, the e-CRFs, the assessment methods, the technical methodology and the basic scientific data. This confidential information will be the property of GEICAM, it must not be disclosed to third parties without the prior written consent of GEICAM and it must not be used other than for the purposes of the study.

The information developed during the practice of this clinical study is also considered to be confidential. This information can be disclosed to the extent considered necessary by GEICAM.

To allow the use of the information derived from this study and to ensure the compliance with the current rules, the investigator is obliged to provide GEICAM with all the results of examinations and all the data developed in this study. Except in that required by law, the information obtained during the study can only be provided to the doctors and to the competent authorities by GEICAM.

GEICAM commits to comply with current legislation relating to studies, which establishes the obligation to publish the results, both positive and negative, in conferences and journals, with reference to the EC/IRB that approved the study, and its funding source. The list of authors will be developed in accordance with the GEICAM SOPs (standard operating procedures). The different disclosures will be decided by the Chief Investigators. By signing this protocol, the Chief Investigators and Principal Investigators accept the terms of the GEICAM's publications policy and commit to respect them.

10.5.1 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations and guidance, GEICAM will, at a minimum register interventional clinical trials sponsored by GEICAM anywhere in the world, on

ClinicalTrials.gov or other publicly accessible websites (European Union Clinical Trials Register and national clinical trials public website (if applicable)), as required by GEICAM policy and local health authorities.

10.5.2 Clinical Trial Results Disclosure

GEICAM will post the results of clinical trials on ClinicalTrials.gov or other publicly accessible websites, as required by GEICAM Policy/Standard and applicable local laws and/or regulations.

10.6. Ethics Committees and/or IRBs

GEICAM or its designee or the Investigator will supply relevant documents for submission to the respective EC/IRB for the protocol's review and approval. This protocol, the Investigator's Brochure/Summary of Product Characteristics [SmPC], a copy of the informed consent form, and, if applicable, subject recruitment materials and/or advertisements and other documents required by all applicable laws and regulations, will be submitted to a central or local EC/IRB for approval. The EC/IRB's written approval of the protocol and subject informed consent must be obtained and submitted to GEICAM or designee before commencement of the study (ie, before shipment of the Sponsor-supplied study treatment or study specific screening activity). GEICAM or its designee will notify the site once GEICAM or its designee has confirmed the adequacy of site regulatory documentation and, when applicable, GEICAM or its designee has received permission from competent authority to begin the trial. Until the site receives notification, no protocol activities, including screening, may occur.

As per applicable regulatory requirements, GEICAM or its designee or the Investigator will submit the required reports of the progress of the study to the EC/IRB and will communicate the possible SAE, the life-threatening adverse events and deaths. At the end of the study, GEICAM or its designee or the Investigator must inform the EC/IRB of trial closure. Al these notifications will be performed according to the applicable regulatory requirements.

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Protocol Attachment 1. Study Schedule

Study Schedule of Events and Timelines. GEICAM/2019-01 (CARABELA)			During Study Treatment. All visits/treatment ± 3 days of scheduled treatment day.				Post-surgery	
Visit	**Baseline**		**Cycle/Visit 1**	**Cycle/Visit 2**	**Subsequent Cycles/Visits**	**Post-treatment visit (from the last study treatment dose)[t,u]**	**Post-surgery visit 30 days (± 7 days) from surgery**	**Follow-up Period every 3 months after Post-surgery visit, during the first 2 years, every 6 months during the following 3 years, and once a year to complete the 10 years FU. (±14 days for the 3-monthly period, ± 28 days for the rest of the visits)**
Day of cycle		RANDOMIZATION	1	1	1			
Procedure/Laboratory/ Diagnostic Test	**Within 28 days (+ 10 days)**							
ICD for Entry (before any study specific tests)[a]	X							
Inclusion/Exclusion Criteria	X							
Medical and surgical history and demographics[b]	X							
Physical examination[c]	X		X	X	X	X	X	

ECOG PS	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Hematology[d]	**X**	**X** **Day 1[f] & 14 (± 1 day) (only Arm B)**	**X** **Day 1 & 14 (± 1 day)(only Arm B)**	**X**	**X**	**X**		
Blood Chemistry[e]	**X**	**X** **Day 1[f] & 14 (± 1 day) (only Arm B)**	**X** **Day 1 & 14 (± 1 day) (only Arm B)**	**X**	**X**	**X**		
Pregnancy test[g]	**X**	**X**	**X**	**X**	**X**			
Standard 12-lead ECG	**X**	**If clinically indicated**						
MUGA or ECHO	**X**			**X[h]**				
Concomitant medications	**X**	**X**						
AEs and SAES[i]	**X**	**X**						
AC followed by Docetaxel/Paclitaxel (Arm A)		**X[j]**	**X**	**X**				
Abemaciclib+Letrozol (Arm B)		**X[k]**	**X**	**X**				
Tumor Assessment	**X[l]**	**Every 12 week (± 1 week) during the treatment phase**			**Prior to surgery**			
Date of death								**X[m]**
Biological samples								
FFPE tumor tissue for central determination of Ki67[n]	**X**							

FFPE tumor tissue for biomarker analysis[o]	**X**		**X** pre-dose and after 2 weeks of treatment (between day 14 and day 21)			**X** (post-treatment biopsy or surgery)		
Tumor tissue for organoids or xenographs generation[p]			**X** pre-dose			**X** (post-treatment biopsy or sugery)		
Blood (plasma and whole blood) samples for ctDNA exploratory biomarker studies[q]			**X** pre-dose and after 2-3 wk		**X** after 12 wk	EOT (prior to surgery)	**X**	X every 6 m during the first five years, every 12 m during the following 5-years and at disease recurrence, whatever occurs first.
Blood (plasma and/or whole blood) samples for other biomarker analysis (e.g. metabolomics and immune response related studies)[r]			**X** pre-dose and after 2-3 wk (local serology report)[s]		X after 12 wk and 6 m both arms (EOT for Arm A)	EOT (prior to surgery)		

Miguel Martín

Study Schedule of Events and Timelines. Protocol GEICAM/2019-01 (CARABELA)

a	Signed, written informed consent (approved by the EC/IRB) obtained prior to any study specific procedure. Informed consent won't be considered as a specific study procedure itself, and may be obtained greater than 28 days prior to the randomization.
b	Includes local laboratory ER/PgR/HER2 expression levels and methods used to assess them, previous treatments. Sex, Race and date of birth.
c	Physical examination includes measurements of height (BL only), weight, body surface area, blood pressure, pulse rate and body temperature. For **Arm A ONLY**: the real Body Surface Area (BSA) of the patient determined in the baseline visit will be the reference BSA throughout the study, except in the event that patients experience body weight variations greater than 10%; in those cases the BSA will be recalculated and the CT drugs dose will be adjusted accordingly. For **Arm B**: the real Body Surface Area (BSA) only will be mandatory at baseline visit.
d	Hemoglobin, WBC, absolute neutrophils, lymphocytes and platelet count. **In the experimental arm (Arm B), the hematology and blood chemistry will be performed every two weeks for the first two visits**(lab test day 14 ± 1 day) and from visit 3 onward, every four weeks (±3 days). In the control arm (Arm A), the hematology and blood chemistry will be performed every three weeks (±3 days). If the patient receives weekly paclitaxel 80 mg/m^2 for 12 weeks, hematology can be performed every 21 days.
e	Fasting glucose, alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine, sodium, potassium, total calcium and urea (or BUN). In the control arm (Arm A), the hematology and blood chemistry will be performed every three weeks (±3 days)**.** If the patient receives weekly paclitaxel 80 mg/m^2 for 12 weeks, blood chemistry can be performed every 21 days.
f	Not on cycle 1, if the assessments were performed within the 7 days previous day 1 of treatment. The initiation of the treatment will be within 5 days after enrollment.
g	Negative serum pregnancy test within 7 days of the first dose of abemaciclib (Arm B) or chemotherapy (Arm A) for premenopausal women, and for women who have experienced menopause onset < 12 month prior to first dose of therapy (CBP women). During treatment phase a **serum or orine pregnancy** test must be performed **in every cycle** and at the **post-treatment visit**, for CBP women, including women with tubal ligation.
h	ECHO or MUGA will be performed at Screening, and for **Arm A after the treatment with anthracyclines**, and if clinically indicated in any treatment arm. It is strongly recommended to use the same method of measurement for the same patient throughout the duration of the study.
i	After informed consent form signature, but prior to initiation of study treatment, only SAEs caused by a protocol-mandated intervention will be collected. Adverse events to be monitored continuously during the treatment period. All AEs occurring during the study and until the treatment discontinuation visit 30 days after the last study medication to be recorded with grading according to NCI-CTCAE, thereafter all study treatment-related SAEs should continue to be collected.
j	Doxorubicin (60mg/m^2) and Cyclophosphamide (600mg/m^2) (AC) iv will be given every 21 days for 4 cycles followed by weekly Paclitaxel (80mg/m^2) for 12 weeks or three-week Docetaxel (100mg/m^2) x 4 cycles. If the patient receives weekly paclitaxel 80 mg/m^2 for 12 weeks, study visits and procedures (ECOG, vital signs, AEs, CM,…) can be performed every 21 days.
k	Letrozole 2.5mg plus Abemaciclib 150mg q12 hours will be taken orally, daily, and continuously for 12 months (±14 days) , the study visits will be performed each 28 (±3) days from first study dose. Premenopausal women must be given LHRH (luteinizing hormone-releasing hormone) analogs.
l	In order to discard metastasis the following tests should be performed before randomization: bone scan if bone pain and/or elevated alkaline phosphatase; abdominal/pelvic computerized tomography scan (CT) if

	elevated alkaline phosphatase, abnormal liver function tests, abdominal symptoms or abnormal physical examination; chest CT if pulmonary symptoms. **Baseline MRI and other diagnostic tests can be performed within 28 days (+ 10 days) prior to randomization.** Tumor evaluation will be done by MRI in baseline and prior to breast surgery, and US will be repeated every 12 week (+/- 1 week) during the treatment phase to discard PD, additional imaging techniques could be done according to investigator's criteria. For **Arm B** MRI could be done from 11 months of study treatment, to allow enough time to plan surgery within 7 days of the last dose of abemaciclib and/or letrozole.
m	The patients will be followed for survival until death, loss to follow-up, withdrawal of consent or study termination by GEICAM. After progression, the tumor assessment will be performed according to the standard medical practice. The date of survival and death (if applicable) will be collected in the eCRF.
n	**A FFPE tumor tissue block for central determination of Ki67** will be collected and sent to the sponsor-designated central laboratories prior to study initiation. Fine needle aspiration will not be allowed. In case of patients with 2 tumor lesions: if both lesions have similar morphological characteristics (i.e. based on local grade, type, Ki67 range…), only the tumor lesion with bigger size will be sent to central laboratory for Ki67 assessment. If tumor lesions have different morphological characteristics, both tumor lesions will be centrally evaluated for Ki67.
o	**FFPE tumor tissue for biomarker analysis** must be collected at baseline (according to the Sample Management Manual, FFPE tumor tissue will be provided from clinical sites to assess a multigene expression platform - OncotypeDx®-; additionally, a core biopsy must be sent to the pathology central lab of the study if there is not enough tumor tissue available after screening)**,** after 2 weeks of treatment (between day 14 and day 21, preferably as close to day 14 as possible) and at EOT (post-treatment core biopsy or surgery block). Fine needle aspiration will not be allowed.
p	**Tumor tissue for generation of organoid-based models and/or xenographs** will be collected in selected sites at baseline and at EOT (post-treatment core biopsy or surgery) . Fine needle aspiration will not be allowed.
q	**Plasma and whole blood for ctDNA analysis:** at baseline (within 7 days prior to initiate study treatment), at 2-3 weeks (wk), at 12 wk (+/- 1 wk), at EOT for any reason (collected within the following 2 wk, prior to surgery), at 4 weeks post-surgery (+/- 2 wk, collected before starting adjuvant CT), every 6 months (+/- 28 days) during the first five years of follow up, every 12 months (+/- 28 days) during the following 5-years and after recurrence, whatever occurs first. EOT means the date of last dose, not the safety visit.
r	**Plasma and whole blood for other biomarker analysis** (e.g. metabolomic and immune response related analysis)**:** pre-treatment (within 7 days prior to initiate study treatment), 2-3 wk, 12-wk (+/- 1 wk), 6 months (+/- 2 wk) in both arms (EOT prior to surgery for Arm A), and EOT prior to surgery for arm B.
s	**A local serology report** (local serology report) performed at any time along the study, but preferably at baseline visit, should be sent to the central laboratory. This serology must include at least HIV, hepatitis B and hepatitis C and, only if available, also hepatitis A, Human Papillomavirus (HPV) and Pox virus.
t	For safety reasons all patients will have a visit after finishing treatment with the study medications. In **Arm A** patients will have this visit not earlier than 21 days after finishing treatment with the study medications and before definitive surgery. In **Arm B** the visit will be done within 7 days from the last abemaciclib and/or letrozole dose In some patients, the end of the study treatment (at neoadjuvant setting) may be due to PD or toxicity. In case the beginning of the new therapy cannot be delayed as per the investigator's judgment, the safety visit may be performed in advance and always before starting the new anticancer therapy.

u	After the last dose of any of the drugs in the NA combinations, in both treatment arms definitive surgery will be performed. For **Arm A** not earlier than 21 days and not later than 42 days after the last dose of chemotherapy, and for **Arm B** within 7 days from the last dose of abemaciclib and/or letrozole, unless toxicities are not recovered completely in any treatment arm.

Protocol Attachment 2. Eastern Cooperative Oncology Group Performance Status

ECOG Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. 1982. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5(6):649-65.

Protocol Attachment 3. Adverse event (AE) non defined in CTCAE

CTC Grade	Equivalent to:	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity.
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the patient.
Grade 3	Severe	Inability to work or perform normal daily activity; treatment or medical intervention is indicated in treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the patient at direct risk.
Grade 4	Life-threatening / disabling	An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing daily activities; treatment or medical intervention is required in order to maintain survival.
Grade 5	Death	AE resulting in death.

Protocol Attachment 4. Adverse Events / Serious Adverse Events Assessment Guide

Time	**After ICD Before Study Treatment**	**During Therapy**	**30-Day** (±7 days) **Post-treatment Follow-up Period**	**Long-Term Follow-up Period**
Events to Collect	AE/SAEs Related to Procedures	New/Ongoing AE/SAEs Regardless of Relatedness to Study Treatment or Procedures		New/Ongoing SAEs Related to Study Treatment or Procedures

Abbreviations: AE = adverse event, ICD = informed consent document, SAE = serious adverse event.

Protocol Attachment 5. List of inducers and strong inhibitors of CYP3A

The information in this list is provided for guidance to investigators and does not preclude the use of these medications if clinically indicated.

Strong inducers

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

Moderate inducers

- Bosentan
- Lenisurad
- Modafinil
- Primidone
- Telotristat ethyl

Strong inhibitors

- Aprepitant
- Ciprofloxacin
- Clarithromycin
- Conivaptan
- Diltiazem
- Erythromycin
- Fluconazole
- Itraconazole
- Ketoconazole
- Nefazodone
- Posaconazole
- Troleandomycin
- Verapamil