Clinical Trial Protocol

A randomized, multicentre, open-label, phase II trial to evaluate the efficacy and safety of palbociclib in combination with fulvestrant or letrozole in patients with HER2 negative, ER+ metastatic breast cancer. (PARSIFAL)

Code: MedOPP067

Study Drug(s):	Palbociclib (PD-0332991) Fulvestrant Letrozole
EudraCT#:	2014-004698-17
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FINAL PROTOCOL APPROVAL

Name	**Date and signature**
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Declaration of Investigators

Protocol Title: A randomized, multicentre, open-label, phase II trial to evaluate the efficacy and safety of palbociclib in combination with fulvestrant or letrozole in patients with HER2 negative, ER+ metastatic breast cancer.

I have received, reviewed and understand the following:

a) Protocol: A randomized, multicentre, open-label, phase II trial to evaluate the efficacy and safety of palbociclib in combination with fulvestrant or letrozole in patients with HER2 negative, ER+ metastatic breast cancer, version dated on 12th December 2019.

b) Investigator's Brochure and SmPC for the IMPs used in the study (Palbociclib, Fulvestrant, Letrozole) with details of clinical and nonclinical data on the investigational product palbociclib (PD-0332991) that are relevant to the study of the product in human subjects

I have been adequately informed about the development of the investigational product to date.

I will confirm the receipt of updated Investigator's Brochure/SmPC

I have read this study protocol and agree that it contains all the information required to conduct the study.

I agree to conduct the study as set out in this protocol.

I fully understand that any changes instituted by the investigator(s) without previous agreement with the sponsor would constitute a violation of the protocol, including any ancillary studies or procedures performed on study patients (other than those procedures necessary for the wellbeing of the patients). I am aware I may only implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favourable opinion and /or before sponsor's agreement and, in this case, and as soon as possible, I will submit in written the implemented deviation or change and the reasons for it to the sponsor.

I will not enrol the first subject in the study until I have received approval from the appropriate Institutional Review Board or Independent Ethics Committee (IRB/IEC) and until all legal and regulatory requirements in my country have been fulfilled.

The study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and its amendments, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines (ICH E6 GCP) and applicable regulations and laws.

I agree to obtain, in the manner described in this protocol and in ICH E6 GCP, written informed consent by the subject or witnessed verbal informed consent to participate for all subjects whose participation in this study is proposed to and before any subject's study specific procedure is done.

I will ensure that the study drug(s) supplied by the sponsor are being used only as described in this protocol. I am aware of the requirements for the correct reporting of serious adverse events, and I commit to document and to report such events as required by the sponsor and in accordance with Health Authority Regulatory requirements.

I agree to supply – upon request – the Sponsor or Sponsor's representative with evidence of current laboratory accreditation, the name and address of the laboratory, and a list of normal values and ranges.
I agree with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals.

I agree to keep all source documents and case report forms as specified in the relevant sections of this protocol.

I will provide all required Regulatory Authority forms, up-to-date curriculum vitae of myself, sub-investigators and of any member of my study team (if requested) before the study starts, which may be submitted to regulatory authorities.

I am aware of the possibility of being audited by the sponsor or its delegate or inspected by regulatory authorities for the performance of this study. I will permit monitoring, auditing and inspection and provide direct access to source data/documents and reports for these purposes.

Furthermore, I confirm herewith that the sponsor is allowed to enter and utilize my professional contact details and function in an electronic database for internal purposes and for submission to Health Authorities worldwide.

Name: _____

Signature: _____ Date: _____

Protocol synopsis

Investigational Medicine Product:	Palbociclib (PD-0332991), Fulvestrant and Letrozole
Protocol Number:	MedOPP067
EudraCT Number:	2014-004698-17
Protocol Title:	A randomized, multicentre, open-label, phase II trial to evaluate the efficacy and safety of palbociclib in combination with fulvestrant or letrozole in patients with HER2 negative, ER+ metastatic breast cancer
Target disease:	ER positive HER2-negative locally advanced or metastatic breast cancer
Subjects:	Postmenopausal women and premenopausal women receiving LHRH analogues, aged ≥ 18 years with ER positive and HER2 negative locally advanced or metastatic breast cancer that had not received any therapy for the metastatic disease. Patients are not eligible if they are candidates for a local treatment with a radical intention. Subjects must have histologic confirmation of the estrogen and/or progesterone-positive and HER2 negative receptors breast cancer. Evidence of measurable or evaluable metastatic disease is required.
Number of patients:	486 patients (anticipated enrolment of 243 patients in Arm A and 243 patients in Arm B).
Selection criteria:	**Inclusion criteria** 1. Postmenopausal women, as defined by any of the following criteria: • Age 60 or over • Age 45 to 59 years and meets ≥ 1 of the following criteria: ➢ Amenorrhea for ≥ 24 months ➢ Amenorrhea for < 24 months and follicle-stimulating hormone within the postmenopausal range (including patients with hysterectomy, prior hormone replacement therapy, or chemotherapy-induced amenorrhea) • Over 18 years of age and bilateral oophorectomy OR Premenopausal women provided they are being treated with LHRH analogues for at least 28 days prior to study entry 2. Eastern Cooperative Oncology Group (ECOG) score lower or equal to 2 3. Histologically confirmed recurrent ER positive (oestrogen and/or progesterone) HER2-negative locally advanced or metastatic BC patients *(Breast cancer that have at least 1% of cells staging positive for ER should be considered ER-positive according to NCCN and ASCO guidelines (1,2))*

4. Patients should not be candidates for a local treatment with a radical intention.
5. No prior hormonal or chemotherapy line in the metastatic setting
6. Patient must have measurable (according to RECIST 1.1) or non measurable disease with these exceptions
    a. Patients with only blastic bone lesions are not eligible
    b. Patients with only pleural, peritoneal or cardiac effusion, or meningeal carcinomatosis are not eligible
7. Life expectancy grater or equal to 12 weeks
8. Adequate organ function:
    a. Hematological: White blood cell (WBC) count >3.0 x 10^9/L, absolute neutrophil count (ANC) >1.5 x 10^9/L, platelet count >75.0 x10^9/L, and hemoglobin >10.0 g/dL (>6.2 mmol/L)
    b. Hepatic: bilirubin < 1.5 times the upper limit of normal (x ULN); alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) <2.5 times ULN. Patients with ALP ≥2.5 times ULN are eligible if ALP abnormalities are unequivocally related to bone lesions (radiological assessments performed within 4 weeks prior to randomization demonstrated bone metastatic disease).
    c. Renal: serum creatinine < 1.5 x ULN.
9. Exhibit patient compliance and geographic proximity that allow for adequate follow-up.
10. Patient has been informed about the nature of study, and has agreed to participate in the study, and signed the Informed Consent form prior to participation in any study-related activities.
11. No other malignancies within the past five years except adequate treated basal cell or squamous cell skin cancer or carcinoma in situ of the cervix
12. Resolution of all acute toxic effects of prior anti-cancer therapy or surgical procedures to NCICTCAE version 4.0 Grade ≤1 (except alopecia or other toxicities not considered a safety risk for the patient at investigator's discretion).
13. Patient has been informed about the translational sub-study and has agreed to participate in the collection of blood and tumor tissue samples by signing the Informed Consent form.

Exclusion criteria

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

1. ER or HER2 unknown disease
2. HER2 positive disease based on local laboratory results (performed by immunohistochemistry/FISH)
3. Locally advanced breast cancer candidate for a radical treatment.

	4. Prior endocrine therapy in the metastatic setting. (Neo)/Adjuvant endocrine therapy is allowed only if the disease-free interval between the end of endocrine therapy and the appearance of metastases in higher than 12 months. 5. Patients with rapidly progressive visceral disease or visceral crisis. 6. Have had a major surgery (defined as requiring general anaesthesia) or significant traumatic injury within 4 weeks of start of study drug, patients who have not recovered from the side effects of any major surgery or patients that may require major surgery during the course of the study. 7. Patients with an active, bleeding diathesis. 8. Have a serious concomitant systemic disorder (e.g. active infection including HIV, or cardiac disease) incompatible with the study (at the discretion of investigator), previous history of bleeding diathesis, or anti-coagulation treatment (The use of low molecular weight heparin is allowed as long as it is used as prophylaxis). 9. Are unable to swallow tablets. 10. History of malabsorption syndrome or other condition that would interfere with enteral absorption. 11. Chronic daily treatment with corticosteroids with a dose of ≥ 10mg/day methylprednisolone equivalent (excluding inhaled steroids). 12. Known active uncontrolled or symptomatic CNS metastases, carcinomatous meningitis, or leptomeningeal disease as indicated by clinical symptoms, cerebral oedema, and/or progressive growth. Patients with a history of CNS metastases or cord compression are eligible if they have been definitively treated with local therapy (e.g., radiotherapy, stereotactic surgery) and are clinically stable off anticonvulsants and steroids for at least 4 weeks before randomization 13. Known hypersensitivity to letrozole, fulvestrant or any of their excipients, or to any PD-0332991 excipients. 14. QTc >480 msec on basal assessments, personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP). 15. Uncontrolled electrolyte disorders that can compound the effects of a QTc-prolonging drug (e.g., hypocalcemia, hypokalemia, hypomagnesemia).
Study objectives	**Primary Objective:** To compare the efficacy of the combination of palbociclib plus fulvestrant versus palbociclib plus letrozole in terms of progression free survival (PFS) in patients with hormone-sensitive HER2-negative metastatic or locally advanced breast cancer. **Secondary Objectives** ✓ To evaluate the safety and tolerability of the combination of palbociclib plus fulvestrant or letrozole.

	✓ To correlate the safety profile of palbociclib combined with fulvestrant or letrozole with baseline patient characteristics. ✓ To compare the time to progression (TTP) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole. ✓ To compare the clinical response (in terms of clinical benefit and overall response) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole. ✓ To compare the duration of response (DoR) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole. ✓ To compare time to response (TTR) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole ✓ To compare the overall survival (OS) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole. ✓ To identify potential biomarkers to predict the benefit from Palbociclib combined with endocrine therapy ✓ To identify mechanisms of resistance to palbociclib combined with endocrine therapy
Type of study:	This is an international, randomized, open-label, controlled, multicenter phase II study. Patients will be stratified by site of disease (visceral vs. non-visceral) and by onset of metastatic disease diagnose (patients metastatic de novo versus non de novo).
Treatment:	Patients will be randomized to one of two therapy regimens. Patients in Arm A will receive fulvestrant 500 mg/5mL i.m. injection administered on Days 1 of a 28-day cycle (loading dose on cycle 1 requires administration also on Day 14). Palbociclib 125 mg capsules will be taken orally once daily beginning on Day 1 of fulvestrant and continuing through Day 21 of every 28-day cycle. Patients in Arm B will receive letrozole 2.5 mg tablet orally once daily beginning on Day 1 and continuing through Day 28 of a 28-day cycle. Palbociclib 125 mg capsules will be taken orally once daily beginning on Day 1 of letrozole and continuing through Day 21 of every 28-day cycle. Patients will continue to receive their assigned treatment until objective disease progression, symptomatic deterioration, unacceptable toxicity, death, or withdrawal of consent, whichever occurs first. Patients discontinuing the active treatment phase will enter a treatment follow-up period during which survival and new anti-cancer therapy information will be collected every 6 months from the last dose of investigational product. The treatment follow-up period will continue up to the end of the study.
Efficacy and Safety assessments	Disease assessments will be performed every 12 weeks (±7 days) from the date of randomization up to end of treatment period. Patients with bone lesions identified at baseline will also have repeat bone scans performed every 24 weeks (±7 days). Each assessment will be performed as scheduled according to the calendar regardless of any dosing delay to prevent the introduction of bias into the assessment of efficacy. Failure to perform any of the required disease assessments will result in the inability to determine disease status for that time point. Tumor assessments will be performed until

	radiographically and/or clinically documented PD as per RECIST v.1.1, study treatment discontinuation, initiation of new anticancer therapy or discontinuation of patient from overall study participation (e.g., death, patient's request, lost to follow-up), whichever occurs first. The occurrence and maximal grade of toxicity for the whole duration of treatment will be listed and tabulated by type and dose level. Adverse events reported as non-drug related by the responsible investigator will be reported as well. Toxicity will be evaluated in this study using the Common Terminology Criteria for Adverse Events by the NCI (CTCAE version 4.0). Blood samples at four time points: baseline, 2 weeks and 12 weeks after treatment start and at time of progression would be collected for translational biomarker research. Additionally, patients must consent to provide tissue samples at baseline and at time of progression (if biopsable lesion). For *non de novo* patients, tumor tissue at the time of metastatic disease diagnose will be requested as preferred option, though at least archived tissue samples from the primary tumor could be acceptable.
Evaluation Criteria	**Primary Endpoint**: The primary endpoint for this study is PFS, which is defined as the time from randomization until objective tumor progression or death, as assessed by the investigator per RECIST v1.1. **Secondary Endpoint-Safety** Patient safety and adverse events will be assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4 [1]. Grade 3 and 4 adverse events and serious adverse events will be assessed to determine the safety and tolerability of the different drug combinations. **Secondary Endpoints-Efficacy** The time to progression (TTP), overall survival (OS), overall response rate (ORR), duration of response (DoR), time to response (TTR) and clinical benefit rate (CBR) will be determined to assess the efficacy of the drug combinations. TTP is defined as the time from randomization to disease progression, as assessed by the investigator per RECIST v1.1. OS is defined as the time from randomization until death from any cause. The ORR is defined as the proportion of patients with best overall response of confirmed complete response (CR) or partial response (PR) based on local investigator's assessment according to RECIST criteria guidelines (version 1.1). DoR is defined as the time from documentation of tumor response (either CR or PR) to disease progression. An objective response needs to be confirmed at least 4 weeks after the initial response. TTR is defined as the time from randomization to the first overall tumor response (tumor shrinkage of ≥30%) observed for patients who achieved a CR or PR. The CBR is defined as the percentage of patients who experience a CR, PR or stable disease for at least 24 weeks and assessed by modified Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1) criteria. **Secondary Endpoints-Translational sub-studies**

	Presence of different pattern of expression of ESR1 mutations and other CDK4/6 related biomarkers (i.e. Rb, Akt, PIK3, p53 CA cyclin D1, cyclin A2, E2F1...) in liquid biopsies and tissue samples. Proteomics analysis to evaluate differential pattern of protein expression from tissue samples. Exome and RNA sequencing will be performed in selected samples
Interim analysis	An interim-analysis will be performed at 22 months after 35% of the total PFS events (89 events) have been observed. Interim analysis will evaluate the primary end-point and all safety and efficacy secondary objectives. Efficacy and safety results of the interim analysis will be evaluated by the Steering Committee that will decide about the suitability of continuing with the study.
Study period	Patients should continue until trial efficacy decision criteria are met. EoS will occur with 254 PFS events and 486 patients included.

Table of abbreviations

Abbreviation	Definition
ABC	Advanced Breast Cancer
ADL	Activities of daily living
AE	Adverse event
AESI	Adverse event of special interest
AI	Aromatase inhibitor
ALT	Alanine transaminase
ANC	Absolute neutrophil count
ALP	Alkaline phosphatase
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
AT	Aminotransferase
AUC	Area under the serum concentration-time curve
BC	Breast cancer
CBR	Clinical benefit rate
CCP	comprehensive cancer panel
CDK	cyclin-dependent kinases
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
Cmax	Maximum concentration
CNS	Central Nervous System
CNV	Copy-number Variation
CR	Complete response
CRF	Case Report Form
CRO	Contract research organization
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DDI	drug-drug interaction
DoR	Duration of Response
DNA	Deoxyribonucleic acid
cfDNA	Circulating free DNA
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
EDTA	ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EoS	End of Study
ER	Endocrine receptors
ESA	Erythropoiesis stimulating agents
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose-Positron Emission Tomography

Abbreviation	Definition
FFPE	Formalin-Fixed, Paraffin-Embedded (tissue)
FISH	Fluorescence in situ hybridization
GCP	Good Clinical Practice
HER2	human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
HR	Hazard Ratio
ICH	International Conference on Harmonization
ILD	Interstitial lung disease
INR	International normalized ratio
IRB	Independent Review Board
ISH	In situ hybridation
ITT	Intention to Treat
LDH	Lactate dehydrogenase
mBC	Metastatic Breast Cancer
MedDRA	Medical Dictionary for Regulatory Activities
MPS	Massive parallel targeted sequencing
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
non-IMP	Non Investigational Medicine Products
OCT	Organic Cation Transporter
ORR	Overall Response Rate
OS	Overall Survival
PCR	Polymerase chain reaction
PD	Progression Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
PP	Protocol compliant population
PPI	proton pump inhibitors
PR	Partial Response
QTcF	corrected QT interval using the Fridericia formula
RBC	Red Blood Cells
RECIST	Response Evaluation Criteria In Solid Tumors
RNA	Ribonucleic acid
miRNA	micro RNA
RP2D	recommended Phase 2 dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Steering Committee

Abbreviation	Definition
SmPC	Summary of Product Characteristics
TdP	Torsade de Pointes
TEAE	Treatment-Emergent Adverse Events
TMA	Tissue microarrays
TTR	Time to Response
ULN	Upper Limit of Normal
UPN	Unique patient number
WBC	White blood cell

Table of contents

I. BACKGROUND AND RATIONALE

1.1. Endocrine therapy in HER2-negative Metastatic Breast Cancer

Breast cancer is the most common tumor among women. In 2012, around 1.7 million new cases were diagnosed with breast cancer around the world. Approximately 232,340 new cases of invasive breast cancer and 39,620 breast cancer deaths are expected to occur among US women in 2013 (3).

The ER+/HER2- population is the largest subgroup among postmenopausal women with metastatic disease representing approximately 65% of cases. Most of these women will receive palliative hormonotherapy as their first approach against the oncological disease. Several large randomized trials have compared the third-generation aromatase inhibitors (letrozole, anastrozole and exemestane) (4–6) (7) to tamoxifen for first-line treatment of metastatic breast cancer (mBC). Overall, these trials have showed that aromatase inhibitors have superior clinical efficacy when compared to tamoxifen and different toxicity profile.

The role of estrogens in breast cancer etiology and progression is well established. Modification of estrogen activity or synthesis represents the treatment of choice for postmenopausal women with hormonal receptor positive advanced breast cancer, particularly for those with slowly progressive disease and limited tumor-related symptoms. Letrozole (Femara®) is an oral non-steroidal aromatase inhibitor and it is approved worldwide for the first-line treatment of postmenopausal women with hormone receptor-positive advanced breast cancer (ABC).

In a multicenter Phase 3 trial, 916 patients with hormone receptor-positive or hormone receptor-unknown ABC were randomized to receive either letrozole or tamoxifen until disease progression. Most of the patients (91%) had received no prior treatment for their advanced disease. Letrozole was superior to tamoxifen for time to tumor progression (median, 9.4 vs. 6.0 months, P<0.0001), time to treatment failure (median, 9 vs. 5.7 months, P<0.0001), overall objective response rate (32%vs21%, P=0.0002), and overall clinical benefit (50% vs. 38%, P=0.0004). Median overall survival (OS) was slightly prolonged for the letrozole arm (34 vs. 30 months); however, approximately 50% of the patients in the tamoxifen arm crossed over to letrozole at disease progression. Letrozole was administered orally on a continuous 2.5 mg daily dosing regimen (5).

Fulvestrant is a novel estrogen-receptor antagonist that, unlike Tamoxifen, is devoid of any agonist activity. After binding to the ER, Fulvestrant induces a rapid degradation and loss of ER and the PgR(8) (9). As a result, there is less chance of the estrogen receptor being activated by alternative pathways that are believed to cause resistance (10).

Fulvestrant has demonstrated similar activity than Tamoxifen when used as initial therapy for the treatment of ER+ metastatic breast cancer progressing on prior endocrine therapy with a clinical benefit rate (CBR) of 58% and a median time to progression (TTP) of 8.2 months. These data supported Fulvestrant approval by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMEA) in this scenario (11).

The combined results of two Phase III clinical trials have shown that Fulvestrant 250 mg had at least the same efficacy results as anastrozole when used in the treatment of patients with advanced breast cancer who had previously progressed on endocrine therapy with a CBR of 43.5% and a median TTP of 5.5 months. These results led to license Fulvestrant as second line endocrine therapy (12).

However pharmacokinetic modelling and evidence of clinical efficacy in early trials, led to suggestions that increasing the Fulvestrant dose would lead to an improved benefit-risk profile. The CONFIRM study is a double-blind, parallel-group, multicenter, phase III trial where more than 700 patients were randomly assigned to fulvestrant 500 mg (500 mg intramuscularly [IM] on day 0, then 500 mg IM on days 14 and 28 and every 28 days thereafter) or 250 mg every 28 days and followed until progression or death. The primary analysis showed a 1-month improvement in median PFS in the group that received the higher dose of Fulvestrant (6.5 versus 5.5 months, HR = 0.80; P=0.006). CBR was 45.6% for fulvestrant 500 mg and 39.6% for fulvestrant 250 mg. A recent release from the study (OS after 75% of the patients had died) has shown a significant gain in survival (OS) for the high dose fulvestrant (HR 0.80, P=.091). The higher dose did not significantly increase the risk of serious adverse events. Based on the results of the CONFIRM trial, whenever Fulvestrant is considered for the treatment of menopausal patients with ER positive advanced BC, the recommended dose is 500mg (13).

Fulvestrant high dose (500 mg monthly) has shown great superiority in a phase II trial (FIRST study) over anastrozole (23.4 vs. 13.1 months, HR 0.66; 95% confidence interval0.47 – 0.92; P = 0.01) (14). The registration phase III trial (FALCON study) is a phase III, randomized, double-blind, multicenter study evaluating the efficacy and safety of fulvestrant 500mg monotherapy compared to anastrozole 1mg as first line treatment for postmenopausal women with hormone receptor-positive locally advanced or mBC

(NCT01602380).. Recently, it has been published the results of FALCON study showing a median PFS in the fulvestrant arm of 16.6 months vs. 13,8 months in the control arm (HR 0.8, [95% CI 0.64-0,99, p = 0.0486). These results confirm the superior efficacy of fulvestrant over anastrozole as first-line treatment in postmenopausal women with hormone receptor-positive locally advanced or mBC.(16)

1.2. Interaction of Estrogens and Cyclin-Dependent Kinases (CDK) in Breast Cancer Cells

Studies of ER-positive breast cancer cell lines indicate that estrogens and antiestrogens act on sensitive populations of cells in early to mid-G1 phase. G1/S transition is under the control of CDKs activated by specific complex formation with regulatory cyclins. CDK4 and CDK6 are activated by binding to D-type cyclins and act early in G1 phase (17)(18) (19) (20).

A primary target of CDK action in G1 phase is the retinoblastoma susceptibility gene product (pRb), which mediates G1 arrest through sequestration of transcriptional factors of the E2F-DP family. Phosphorylation of pRb and other members of the pocket protein family (p107 and p130) by active cyclin-CDK complexes leads to release of E2F and DP transcription factors and transcription of requisite genes for S-phase entry.10 D-type cyclins play an essential role in recognition of extracellular growth stimuli and initiation of G1 transit(21)(22) and several lines of evidence have linked estrogen regulation of cellular proliferation to cyclin D1 expression. Estrogen-induced proliferation of normal uterine and breast epithelium in vivo is associated with increased expression of cyclin D1 mRNA and protein (23)(24)(25)(26). Expression of cyclin D1 in breast tumor isolates correlates with ER-positive status (27)(28)(29).

Antiestrogen-induced growth arrest of ER-positive breast cancer cells is associated with decreased cyclin D1 expression (30). These studies are consistent with a model of estrogen action in which receptor activation induces increased cyclin D1 expression, CDK4 activation, and cell cycle progression. An upstream role for cyclin D1 has been suggested by recent reports describing direct physical interactions between cyclin D1 and the ER, leading to recruitment of steroid receptor coactivators and activation of ER-dependent transcription. This occurs in the absence of hormone and is independent of D-type cyclin association with CDK4 (31)(32)(33)(34). Constraint upon CDK activity and G1 progression is provided by the universal CDK inhibitors of the Cip-Kip family, including gp21Cip1 and p27Kip1, and the specific CDK4 and CDK6 inhibitors of the INK4

family, typified by p16INK4a (22)(35)(36)(37)(38). Functional association of cyclin D1-CDK4 is required for estrogen-induced CDK2 activation and G1/S transition and estrogen regulates expression of p21Cip1, p27Kip1, and Cdc25A independent of D-type cyclin-CDK4 function (39).

1.3. Palbociclib (PD-0332991): Selective inhibitor of cyclin dependent kinases 4 and 6 (CDK4/6)

Palbociclib (PD-0332991) is an oral and selective inhibitor of cyclin dependent kinases 4 and 6 (CDK4/6) with little or no activity against a large panel of 274 other protein kinases. The only known natural substrate for Cdk4/cyclin D1 is the retinoblastoma gene product, Rb. Palbociclib has shown to have no effect in Rb-negative tumor cells.

Therefore, the phosphorylation status of Rb in treated tumors can serve as a biomarker for target modulation by palbociclib. CDK4/6 are involved in cell progression during phase G1 to phase S. Inhibition of both: CDK4 and 6 has been shown to prevent the deactivation of retinoblastoma susceptibility gene protein and interfere with tumor cell progression. In preclinical studies it has been shown that palbociclib inhibits cell growth and DNA replication through preventing cells from entering S phase. Palbociclib inhibited thymidine incorporation into the DNA of a panel of Rb-positive human breast, colon, and lung carcinomas as well as in leukemias and in non-transformed human epithelial cells and fibroblasts.

Preclinical data for Palbociclib

Data form preclinical studies indicate that palbociclib may have cytoreductive as well as cytostatic effects on tumor cells. In *in vivo* studies the MTD in mice was determined to be 150mg/kg/day during 14 days. At this regimen palbociclib had significant antitumor efficacy against multiple human tumor xenograft models (40). Again it was confirmed that Rb-negative tumors do not respond to palbociclib. The lack of efficacy in these cases is consistent with the lack of anti-proliferative activity observed *in vitro* (41).

Taken together, these results support the proposed mechanism of palbociclib and the specificity of the compound demonstrated in enzyme activity test.

Further studies investigated whether continuous daily dosing of palbociclib was needed for optimal efficacy. The results in breast and colon carcinoma models showed that a

similar degree of efficacy was attained among several schedules of treatment, implying that an intermittent regimen is feasible without compromising activity (42) (43).

The primary toxicities observed in preclinical studies were to the hematolymphopoietic tissues and male reproductive organs, and the potential for QT prolongation and developmental toxicity. Palbociclib was also identified as an aneugen.

Summary of safety and efficacy clinical data for palbociclib

At least 15 studies have evaluated the safety, efficacy, pharmacodynamics and pharmacokinetics of palbociclib (as single agent or in combination) in solid tumors, multiple myeloma, lymphomas and breast cancer (44)(45). As of December 2014 cut-off date, 5 clinical studies in patients with palbociclib are on-going/initiated and 3 clinical studies have completed. Additionally, palbociclib has been investigated in 15 completed and 2 on-going Phase I clinical pharmacology studies in healthy volunteers. Four hundred healthy volunteers have received single doses of palbociclib ranging from 50 mg to 150 mg, and twenty-six healthy volunteers have received multiple doses of palbociclib on a 125 mg QD regimen.

The phase I dose-escalation study A5481001 evaluated two different schedules of palbociclib: Schedule 3/1 or 2/1 (two weeks on and 1 week off)(41). Overall the adverse events were manageable and reversible. The dose limiting toxicities were similar between both schedules and consisted primarily of myelotoxic events. A clear dose relationship was evident.

With the recommended phase 2 dose on the 21-day schedule (125mg/day), 3 of 22 patients had grade 3 toxic effects during the first cycle, but none had grade 4 toxicity. As for the 14-day schedule (with 200mg/day): 6 of 20 patients had grade 3 toxic effects but 3 of 6 had grade 3/4 toxic effects at the immediately higher dose level.

The most frequently reported treatment-emergent adverse events (TEAEs) of any grade were neutropenia (46%) fatigue (45%) diarrhoea, anaemia and nausea (25%). The most frequently reported grade 4 TEAEs was neutropenia (6.6%), thrombocytopenia (3.3%) leukopenia (2.2%) and anaemia (1%). No episodes of febrile neutropenia were observed. Neutropenia and thrombocytopenia were dose dependent with both nadirs observed at the end of the dose period.

Therapeutic benefit was observed with both treatments schedules: On the 21-day schedule, 35% patients achieved stable disease for at least 2 cycles, of whom 6 had

stable disease for 10 or more cycles at doses ranging from 50 to 150 mg/d. On the 14-day schedule, 1 patient had a partial response, 9 patients (29%) experienced stable disease lasting at least 2 cycles, and 3 patients (10%) had stable disease for 10 or more cycles at the 200-mg/d dose level. Greater long-term antitumor activity was observed with schedule 3/1, therefore this regimen was selected for further clinical development. The recommended phase II dose (RP2D) was 125 mg QD and 200mg QD respectively.

In the phase II portion of the study A5481003 (phase I/2 study) patients were randomized to receive letrozole monotherapy or letrozole plus palbociclib. The most frequent TEAEs (≥20% of patients) any grade related to the study treatment were: neutropenia (72%), fatigue (28%), leukopenia (42%) and anaemia (28%). Grade 3 Neutropenia was present in more than 40% of patients, and leukopenia grade 3 in more than 20%.

According with the toxicity observed in the previous phase I and phase II trials in the phase III study (A5481008) the most frequently reported grade 3 TEAEs were neutropenia (12%) and neutrophil count decreased (less than 10%) (46).

A total of 400 healthy volunteers received single doses of palbociclib, ranging from 50 mg to 150 mg, as of the December 2014 cut off date.

The most frequently reported TEAEs of any grade, considered related to study treatment were headache (5.0%), nausea (2.8%), somnolence (2.3%) and abdominal distension (2.0%). No severe AEs, no serious AEs and no deaths have been reported from these studies.

In summary, it can be concluded that the typical palbociclib toxicity is bone marrow toxicity that is generally manageable, predictable, reversible and dose dependent.

Although phase I studies were not designed with a primary endpoint to test efficacy, response rate were evaluated. Stable disease lasting ≥2 cycles were observed in about 30% of patients. In the A5481003 (PALOMA-1) the response data from the phase I portion of the study showed that 33% of patients achieved a PR and another 42% of patients had stable diseases for ≥ 6 months. The interim efficacy results form part 1 of the phase II portion of study A5481003 showed that the median progression-free survival was longer in the combination group (18.2 months vs. 5.7 months) as well as the clinical benefit rate (76 vs. 47%) was (47).

More mature data from this study were reported later in the 2012 CTRC- San Antonio Breast Cancer Symposium and thereafter in the AACR (American Association for cancer research) Annual Meeting 2014. The final efficacy results from the Phase II portion of the

study showed that the median progression-free survival was significantly longer in the palbociclib plus letrozole arm than in the letrozole alone arm (20.2 months vs. 10.2 months, respectively) with a hazard ratio of 0.488 (95% CI: 0.319-0.748) in favour of palbociclib plus letrozole (p=0.0004).

The combination was also associated with a higher objective response rate and clinical benefit rate than letrozole alone (43% vs. 33% and 81% vs. 58%, respectively [in the intent-to-treat population based on investigator assessment]).

Palbociclib has been authorized for marketing in the U.S. On 03 February 2015 the U.S. Food and Drug Administration (FDA) approved palbociclib capsules in combination with letrozole for the treatment of postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer as initial endocrine-based therapy for their metastatic disease.

1.4. ***Rationale for combining letrozole with palbociclib***

Preliminary data of the randomized phase II study PALOMA-1 (also known as Study 1003) were presented at the 2012 CTRC- San Antonio Breast Cancer Symposium. In this trial women treated with the combination of palbociclib (125mg once daily for three out of four weeks in repeated cycles) + letrozole (letrozole 2.5mg once daily on a continuous regimen) achieved a statistically significant improvement in terms of median progression free survival (PFS) compared to women in the control arm (26.1 months vs. 7.5 months) (48). Based on these data, palbociclib received the Breakthrough Therapy designation by the United States Food and Drug Administration (FDA). The updates of these data were recently reported in the AACR (American Association for Cancer Research) Annual Meeting 2014. Women receiving palbociclib + letrozole obtained a doubled median of progression-free survival when compared with women in the control arm (20.2 months vs. 10.2 months; HR = 0.488, 95%CI: 0.319 – 0.748, p=0.0004) and a trend toward increase overall survival (37.5 months vs. 33.3 months; HR = 0.813, 95% CI 0.492 – 1.345). The progression free survival results indicated a 51% reduction in the risk of disease progression with the addition of palbociclib. The combination arm was well-tolerated and the safety profile of the combination was consistent with previously reported data. The most common adverse events in the palbociclib arm were neutropenia, leukopenia, fatigue and anemia.

The PALOMA-1 consisted in two different parts: part 1 enrolled 66 patients with ER+HER2- mBC, part 2 enrolled 99 additional patients whose tumors presented two

biomarkers: cyclin D1 amplification and/or p16 loss. The risk for progression of disease did not decrease further in patients whose tumors had the molecular targets specific for the drug.

The PALOMA-2 study is a randomized (2:1), multicenter, double blind, placebo controlled phase III trial that evaluates palbociclib in combination with letrozole versus letrozole plus placebo as first-line treatment for post-menopausal patients with ER+/HER2- locally advanced or mBC patients (46). Recent results showed that the trial met its primary end-point, median PFS in women treated with the combination resulted in an improvement of 10 months in terms of PFS: 24,8 vs. 14,5 months in the control arm (HR 0.59; p <.00001) (49).

1.5. Rational for combining palbociclib with fulvestrant

In the PALOMA-3 study, the combination of palbociclib and fulvestrant was associated with significant improvements in progression-free survival compared with fulvestrant plus placebo in patients with metastatic breast cancer with disease relapse or progression after previous endocrine therapy for advanced disease . Median PFS was 9·5 months (95% CI 9·2-11·0) in the fulvestrant plus palbociclib group and 4·6 months (3·5-5·6) in the control arm (fulvestrant monotherapy) (HR 0·46, 95% CI 0·36-0·59, p<0·0001). (50)

Grade 3 or 4 adverse events occurred in 251 (73%) of 345 patients in the fulvestrant plus palbociclib group and 38 (22%) of 172 patients in the fulvestrant plus placebo group. The most common grade 3 or 4 adverse events were neutropenia (223 [65%] in the fulvestrant plus palbociclib group and one [1%] in the fulvestrant plus placebo group), anaemia (ten [3%] and three [2%]), and leucopenia (95 [28%] and two [1%]). Serious adverse events (all causalities) occurred in 44 patients (13%) of 345 in the fulvestrant plus palbociclib group and 30 (17%) of 172 patients in the fulvestrant plus placebo group.

Results from PALOMA-3 trial have supported the expanded indication of palbociclib to be used in combination with fulvestrant for treatment of women with HR+/HER2 advanced or metastatic breast cancer (MBC) whose cancer has progressed after endocrine therapy.

1.6. Rational of the study

Palbociclib registration program includes two phase III trials evaluating palbociclib in combination with endocrine therapies in post-menopausal patients with ER+/HER2- locally advanced or mBC. The PALOMA-2 trial compares letrozole plus palbociclib to

single agent letrozole as first line therapy. The PALOMA-3 evaluates the combination of fulvestrant and palbociclib to single agent fulvestrant on patients with mBC who progressed to an aromatase inhibitor. Both trials are designed to demonstrate superiority of the combination arms in terms of progression free survival. However, even if the design of both trials is very similar, differing just on the line of therapy (first vs. second), their biological assumptions are largely different. While the front line PALOMA-2 trial has a solid backbone coming from the PALOMA-1 trial as well as preclinical models, PALOMA-3 relies on the assumption that the mechanisms of resistance to aromatase inhibitors are not driven or will overlap the cyclin machinery (51).

On the other hand, FIRST, the phase II study that compared fulvestrant at optimal doses (fulvestrant high dose or fulvestrant HD) to anastrozole as front line therapy for ER-positive mBC, provided robust data in favour of fulvestrant in terms of PFS. Recently, it has been announced that the registration phase III study (FALCON) that compares fulvestrant to anastrozole as first line treatment for postmenopausal women with no prior endocrine therapy has met its primary end-point confirming the superiority of fulvestrant HD vs. anastrozole in terms of PFS. However, median PFS in the fulvestrant arm (16.6 months) though statistically superior to its control arm is inferior to the one achieved in the phase II FIRST study (23.4 months).

An indirect comparison between data available for palbociclib (PALOMA- 1 and PALOMA-2) and fulvestrant HD (FIRST and FALCON) identifies favourable Hazard Ratios for letrozole-palbociclib and fulvestrant HD compared with standard aromatase inhibitors as front line endocrine therapy for ER-positive locally advanced or mBC.

With two new but different standard of care as front line endocrine therapy for advanced breast cancer, exploring the combination of palbociclib plus fulvestrant in the front line setting seems mandatory, especially if preclinical data concerning mechanisms of resistance are confirmed.

The present study (PARSIFAL) is an open-label, randomized, controlled, multicenter phase II study with the aim of assessing the efficacy and safety of the combination palbociclib plus fulvestrant vs. palbociclib plus letrozole in terms of PFS in women with ER-positive advanced BC.

1.7. Rational of molecular part of the study

Estrogen Receptor is the primary therapeutic target in breast cancer and is overexpressed in 70% of cases. However, approximately 30% of ER-positive breast cancers exhibit de novo resistance, whereas 40% acquire resistance to these therapies. In addition to antiestrogen therapies, patients with ER-positive breast cancer are treated with aromatase inhibitors such as letrozole and exemestane. As with anti-estrogens, treatment with aromatase inhibitors results in the development of resistance, but this is presumably due to different mechanisms, as patients with breast cancer who develop resistance to aromatase inhibitors often still respond to anti-estrogen therapies. The molecular mechanisms of endocrine resistance in ER-positive breast cancer continue to be an active area of research.

One of the best defined mechanisms of resistance to endocrine therapy in breast cancer is increased expression of genes related to proliferation, including cyclin D1 and genes regulated by MYC, E2F, and RB. These findings provided a strong justification to target proteins involved in the G1–S cell-cycle transition like cyclin CDK4/6 in order to overcome resistance.

Palbociclib is a new drug that targets CDK4/6. Preclinical studies have identified the luminal estrogen receptor (ER) subtype, elevated expression of cyclin D1 and Rb proteins, and reduced p16 expression as associated with sensitivity to palbociclib. In the clinical setting, CCND1 amplification and/or loss of p16 were investigated as potential predictors of palbociclib sensitivity in the PALOMA-1 trial with little success (52). If little is known about markers of sensitivity to palbociclib, the lack of knowledge of the mechanisms that drive resistance is even greater and most of the studies in this area have been performed in preclinical models. In breast cancer cell lines it has been demonstrated that the chronic loss of Rb is specifically associated with evolution to a CDK4/6-independent state and, ultimately, resistance to palbociclib (53). On the other hand, models of resistance that retain Rb function are related to elevated expression of E2F target genes (CCNE1, CCNA2 and E2F1); and this elevated E2F signalling still shows sensitivity to palbociclib on a reduced magnitude. Finally, another suggested mechanism of palbocicib resistance is Cyclin E1 overexpression (54).

The PARSIFAL trial offers an unique opportunity to gain insight in the mechanisms responsible for the acquisition of resistance to endocrine therapy and palbociclib in the clinical setting. The prognostic and predictive value of a variety of biomarkers will be

evaluated, with particular emphasis in mutations, amplifications and deletions, and expression profiles of selected genes.

Intratumor genetic heterogeneity and clonal evolution/selection during therapy significantly compromise the usefulness of primary tumour tissue as a source to study predictive biomarkers for metastatic disease (55). For example, ESR1 mutations are rarely found within primary tumour tissue, even by means of ultra-deep sequencing, but are present in up to 20% of ER-positive metastases and confer resistance to estrogen deprivation, potentially increasing with the number of lines of treatment in this situation with a very low rate of mutation in non metastatic situation (56). These mutations seem to confer resistance to certain endocrine treatments, including aromatase inhibitors or tamoxifen, while fulvestrant might be effective in a dose dependant relation (57). Furthermore, recently published in vitro data also suggest a lack of sensitivity of ER mutated cell clones to CDK4/6 inhibitors such as palbociclib (58).

Liquid biopsies allow the detection of mutations present within the primary tumour but also those which are exclusive to metastases (59). Also, the usefulness of circulating free DNA (cfDNA) for mutational analysis in breast cancer has recently been demonstrated (60). In view of this evidence, within the PARSIFAL study blood will be sequentially collected from patients and circulating free DNA (ctDNA) will be isolated () and used to monitor mutations However, due to the extreme fragmentation of cfDNA and the fact that mutated alleles are greatly diluted within a dominating population of “normal”, non-tumour derived cfDNA, genetic analysis in this setting remains technically challenging and it is not yet possible to investigate amplifications and deletions. In contrast, plasma has been demonstrate to constitute a useful source of material for RNA purification and gene expression analyses at least in some malignancies (61).

Molecular alterations at the genomic as well as transcriptional level, including amplifications/deletions and gene expression profiles yield a wealth of potential predictive biomarkers Therefore the analysis of metastatic tissue to identify predictive biomarkers still remains indispensable even if this constitutes a more invasive and less convenient method than liquid biopsy (62)(63)(64)

2. OBJECTIVES and ENDPOINTS

2.1. Primary Objective:

To compare the efficacy of the combination of palbociclib plus fulvestrant versus palbociclib plus letrozole in terms of progression free survival (PFS) in patients with hormone-sensitive HER2-negative metastatic or locally advanced breast cancer.

Primary Endpoint: to evaluate the progression-free survival. This is defined as the time from randomization until objective tumor progression or death by any cause.

2.2. Secondary objectives:

- To evaluate the safety and tolerability of the combination of palbociclib plus fulvestrant or letrozole.
- To correlate the safety profile of palbociclib combined with fulvestrant or letrozole with baseline patient characteristics.
- To compare the time to progression (TTP) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole.To compare the clinical response (in terms of clinical benefit and overall response) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole.
- To compare the duration of response (DoR) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole.
- To compare time to response (TTR) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole.
- To compare the overall survival (OS) of the combination of palbociclib plus fulvestrant with palbociclib plus letrozole.
- To identify potential biomarkers to predict the benefit from Palbociclib combined with endocrine therapy
- To identify mechanisms of resistance to palbociclib combined with endocrine therapy

Secondary Endpoint-Safety: Patient safety and adverse events will be assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4[1]. Grade 3 and 4 adverse events and serious adverse events will be assessed to determine the safety and tolerability of the different drug combinations.

Secondary Endpoints-Efficacy:

- ✓ The Time to progression (TTP), defined as the time from randomization to disease progression, as assessed by the investigator per RECIST v1.1.
- ✓ The overall response rate (ORR), defined as the proportion of patients with best overall response of confirmed complete response (CR) or partial response (PR) based on local investigator's assessment according to RECIST criteria guidelines (version 1.1) (65). An objective response needs to be confirmed at least 4 weeks after the initial response.
- ✓ Duration of response (DoR) is defined as the time from documentation of tumor response (either CR or PR) to disease progression.
- ✓ TTR is defined as the time from randomization to the first overall tumor response (tumor shrinkage of ≥30%) observed for patients who achieved a CR or PR.
- ✓ Clinical Benefit Rate (CBR) defined as the percentage of patients who experience a CR, PR or stable disease (for at least 24 weeks) and assessed by modified Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1) criteria.
- ✓ The overall survival (OS), defined as the time from randomization until death from any cause.

Secondary Endpoints-Translational sub-studies

- ✓ Presence of different pattern of expression of ESR1 mutations and other CDK4/6 related biomarkers (i.e. Rb, Akt, PIK3, p53 CA cyclin D1, cyclin A2, E2F1...) in liquid biopsies and tissue samples.
- ✓ Proteomics analysis to evaluate differential pattern of protein expression from tissue samples.
- ✓ Exome and RNA sequencing will be performed in selected samples

3. STUDY OVERVIEW

3.1. Study Design

This is an international, randomized, open-label, controlled, multicenter phase II clinical trial to investigate and compare the safety and efficacy of palbociclib combined with fulvestrant or letrozole in women with ER+, HER2- locally advanced or metastatic breast cancer.

Eligible patients will have histologically proven diagnosis of adenocarcinoma of the breast with evidence of locoregionally recurrent or metastatic disease and will be candidates to receive letrozole as first-line treatment for their advanced disease. Patients could not be eligible if they are candidates for a local treatment with a radical intention. In order to avoid inclusion of patients who are refractory or resistant to endocrine therapy, patients who received endocrine therapy as a component of their (neo)adjuvant regimen may only enter the study if their disease did not progress while on or within 12 months from completion of their endocrine-containing (neo)adjuvant therapy. Patients should not have received endocrine therapy or prior chemotherapy for their advanced disease. Patients can have measurable disease as per RECIST v.1.1 or non-measurable disease (except for patients with only blastic bone lesions, patients with only pleural, peritoneal or cardiac effusion, or meningeal carcinomatosis).

At least 486 eligible patients will be randomized 1:1 to receive either palbociclib (PD-0332991) plus fulvestrant (Arm A: at least 243 patients) or palbociclib (PD-0332991) plus letrozole (Arm B: at least 243 patients).

Patients will be stratified by site of disease (visceral[1] vs. non-visceral[2]), and by the onset of metastatic disease diagnose (de novo metastatic versus non de novo patients).

3.2. Study treatment management

After signing the informed consent form, patients will be randomized (1:1) to one of two therapy regimens:

- Patients in Arm A will receive fulvestrant 500 mg/5mL i.m. injection administered on Days 1 and 14, of a 28-day cycle on cycle one and one monthly thereafter.

[1] *Visceral: refers to any lung (including pleura) and/or liver involvement*

[2] *Non-visceral: refers to absence of lung (including pleura) and/or liver involvement*

Palbociclib 125 mg total dose will be taken orally once daily beginning on Day 1 of fulvestrant and continuing throughout Day 21 of every 28-day cycle.

- Patients in Arm B will receive letrozole 2.5 mg orally once daily beginning on Day 1 and continuing through Day 28 of a 28-day cycle. Palbociclib 125 mg capsules will be taken orally once daily beginning on Day 1 of letrozole and continuing throughout Day 21 of every 28-day cycle.

Patients will continue to receive their assigned treatment until objective disease progression, symptomatic deterioration, unacceptable toxicity, death, or withdrawal of consent, whichever occurs first.

All patients who have not progressed and are still in receipt of study treatment at the end of the study (EoS), as defined here in section 3.7, at the discretion of the investigator might continue to receive the study treatment. In this case, the investigator should follow the patient appropriately as per standard clinical practice. Sponsor supply for study IMPs will continue after the end of the study if IMPs are not available in a reimbursed setting.

Patients discontinuing the study treatment period will enter a treatment follow-up period during which survival and new anti-cancer therapy information will be collected every 6 months from the last dose of investigational product. The study follow-up period will conclude when trial efficacy decision criteria are met. The final analysis should occur with 254 PFS events and 486 patients included. Efficacy analyses will be performed using the local radiologist's/investigator's tumour assessments as primary data source.

3.3. General Concomitant Medication and Supportive Care Guidelines

Concomitant therapy and pre-medications are defined as non-IMPs. Concomitant therapy includes any prescription medication, over-the-counter preparation, or herbal therapy between the 21 days preceding first treatment and the safety follow up visit (28 +/- 7 days of last IMP administration). All concomitant therapies will be registered until the safety follow-up visit. Afterwards, only information about further anti-cancer therapies received by the patient once she goes off study will be collected.

All concomitant medications will include the start date, stop date, brand or generic name, route of administration, dose and indication for the treatment.

No pre-medication for any study arm is required; however, pre-medication is allowed at the investigator's discretion.

The main target organ for toxicity is bone marrow. Erythropoiesis stimulating agents (ESAs) (such as Procrit®, Aranesp®, Epogen®) may be used for supportive care according to recommendations of National Comprehensive Cancer Network (NCCN) guidelines. Granulocyte-colony stimulating factors should not be used prophylactically but they may be used in treatment-emergent neutropenia in accordance NCCN guidelines.

Once the patient is on study treatment, palliative radiotherapy may be permitted to treat pre-existing painful bone metastases or to treat brain metastases (for patients who have disease control outside of the brain). Please contact the Medical Monitor for approval.

Other medications considered necessary for the patient's safety and wellbeing may be given at the discretion of the investigator. Use of bisphosphonates or denosumab is permitted for the control of bone pain, prevention and/or treatment of bone metastases, and treatment of osteoporosis. If bisphosphonates are required either for the treatment of symptomatic malignancy-associated hypercalcemia or for pain control, tumor assessments should be performed to assess for potential disease progression.

3.4. Prohibited therapies

Use of the therapies described below is prohibited during the study prior to discontinuation of study treatment (collectively, these will be referred to as non-protocol therapy):

Any therapies intended for the treatment of cancer, other than palbociclib, fulvestrant or letrozole whether they are approved by national health authorities or experimental, including cytotoxic chemotherapy, immunotherapy, hormonal therapy, and biologic or targeted agents are prohibited.

Palbociclib is primarily metabolized by CYP3A4 enzymes, the concurrent use of strong CYP3A inhibitors or inducers may change the plasma concentrations of palbociclib. For this reason the concurrent use of strong CYP3A inhibitors or inducers should be avoided. Strong inhibitors of CYP3A include but are not limited to boceprevir, clarithromycin, conivaptan, delavirdine,, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, posaconazole, lopinavir/ritonavir, saquinavir, telaprevir, telithromycin, voriconazole, grapefruit juice and grapefruit. Strong inducers of CYP3A

include but are not limited to carbamazepine, enzalutamide, felbamate, nevirapine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentin, and St. John's Wort Coadministration of moderate CYP3A inducers may also decrease the plasma exposure of palbociclib. Moderate CYP3A inducers including, but not limited to, bosentan, efavirenz, etravirine, modafinil, and nafcillin can be used concurrently with palbociclib when it cannot be avoided. No dosing adjustments are required.

Palbociclib is a weak time -dependent inhibitor of CYP3A following daily 125 mg dosing to steady state in humans. The dose of the sensitive CYP3A substrate with a narrow therapeutic index (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus and tacrolimus) may need to be reduced as palbociclib may increase their exposure.

Radiotherapy for unequivocal disease progression is not permitted while on study treatment, with the following exception of new central nervous system (CNS) metastases or isolated progression of previously treated CNS lesions. Patients who have disease control outside of the CNS, defined as confirmed PR or CR of any duration, or SD for ≥ 3 months, but who have developed CNS metastases that are treatable with radiation will be allowed to continue to receive study therapy until they either experience systemic progression of their disease outside of the CNS and/or further progression in the CNS that cannot be treated with additional radiation. Patients must not miss more than one cycle of study treatment for the treatment of their CNS metastases and must have an ECOG performance status of 0, 1, or 2 to continue on study treatment. The Medical Monitor should be informed before a decision is made to resume study treatment after radiotherapy for CNS metastases.

Drugs known to cause QT interval prolongation are also prohibited during the active treatment phase.

In general, the following treatments are not prohibited but they are not recommended during the treatment phase:

- Chronic immunosuppressive therapies 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 if daily dose is below of 10 mg of methylprednisolone equivalent.
- Herbal medicine.

3.5. Duration of study treatment

The study treatment period is defined as the time between the study entry (date of randomization) and the last dose of interventional arm (Arm A) or control arm (Arm B) regimens.

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue with the protocol therapy, the study treatment should be discontinued and the reason(s) for discontinuation documented in the clinical records of the patient and corresponding case report form.

Study treatment may continue until one of the following criteria applies:

- Radiologically confirmed and documented unequivocal disease progression, with the following exception of new CNS metastases or isolated progression of previously treated CNS lesions. Patients who have disease control outside of the CNS, defined as confirmed PR or CR of any duration, or SD for ≥ 3 months, but who have developed CNS metastases that are treatable with radiation will be allowed to continue to receive study therapy until they either experience systemic progression of their disease outside of the CNS and/or further progression in the CNS that cannot be treated with additional radiation
- Adverse event(s) that according to the protocol or in the judgment of the investigator may cause severe or permanent harm or which rule out continuation of study drug. *Note: see detailed criteria for study treatment discontinuation due to toxicity in section* 7.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Serious non-compliance with the study protocol.
- Investigator removes the patient from study.
- Death.
- Lost to follow-up.
- Patient withdraws consent.
- The study site or the sponsor decides to close the study.

All patients who have not progressed and are still in receipt of study treatment at the end of the study (EoS), as defined here in section 3.7, at the discretion of the investigator might continue to receive the study treatment. In this case, the investigator should follow the patient appropriately as per standard clinical practice. Sponsor supply for study IMPs will continue after the end of the study if IMPs are not available in a reimbursed setting.

3.6. Duration of treatment follow-up period

Patients should continue until trial efficacy decision criteria are met. The final analysis should occur with 254 PFS events and 486 patients included.

3.7. End of Study (EoS)

EoS will be achieved when trial efficacy decision criteria are met (final analysis expected with 254 PFS events and 486 patients included), unless premature termination of the study. This will be the last data collection point, which can be a clinic visit or a laboratory sample.

Patients discontinuing the study should be followed-up as per standard clinical practice.

4. PATIENT SELECTION

This study can fulfil its objectives only if appropriate patients are enrolled. The following eligibility criteria are designed to select patients for whom the protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular patient.

4.1. Target study population

This study will enrol postmenopausal patients or pre-menopausal patients under treatment with LHRH analogues with histologic confirmation of the ER (oestrogen and/or progesterone-positive) and HER2-negative locally advanced or metastatic BC. Breast cancer that have at least 1% of cells staging positive for ER should be considered ER-positive according to NCCN and ASCO guidelines (1)(2). Evidence of measurable disease (as for RECIST 1.1) or non-measurable disease is required (Patients with only blastic bone lesions, only pleural, cardiac or peritoneal effusion or meningeal carcinomatosis are not eligible. Patients are not eligible if they are candidates for a local treatment with a radical intention.

4.2. Inclusion criteria

Patient eligibility should be reviewed and documented by an appropriate member of the investigator's study team before patients are included in the study.

Patients must meet all of the following inclusion criteria to be eligible for enrolment into the study:

1. Postmenopausal women, as defined by any of the following criteria:

- Age 60 or over
- Age 45 to 59 years and meets ≥ 1 of the following criteria:
    - Amenorrhea for ≥ 24 months
    - Amenorrhea for < 24 months and follicle-stimulating hormone within the postmenopausal range (including patients with hysterectomy, prior hormone replacement therapy, or chemotherapy-induced amenorrhea)
- Over 18 years of age and bilateral oophorectomy

OR

Premenopausal women provided they are being treated with LHRH analogues for at least 28 days prior to study entry

2. Eastern Cooperative Oncology Group (ECOG) score lower or equal to 2
3. Histologically confirmed recurrent ER positive (oestrogen and/or progesterone) HER2-negative locally advanced or metastatic BC patients (Breast cancer that have at least 1% of cells staging positive for ER should be considered ER-positive according to NCCN and ASCO guidelines (1,2))
4. Patients should not be candidates for a local treatment with a radical intention.
5. No prior hormonal or chemotherapy line in the metastatic setting
6. Patient must have measurable (according to RECIST 1.1) or non measurable disease with these exceptions
    - Patients with only blastic bone lesions are not eligible
    - Patients with only pleural, peritoneal or cardiac effusion, or meningeal carcinomatosis are not eligible
7. Life expectancy greater or equal to 12 weeks
8. Adequate organ function:
    - Hematological: White blood cell (WBC) count >3.0 x 109/L, absolute neutrophil count (ANC) >1.5 x 109/L, platelet count >75.0 x109/L, and hemoglobin >10.0 g/dL (>6.2 mmol/L)
    - Hepatic: bilirubin < 1.5 times the upper limit of normal (x ULN); alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) <2.5 times ULN. Patients with ALP ≥2.5 times ULN are eligible if ALP abnormalities are unequivocally related to bone lesions (radiological assessments performed within 4 weeks prior to randomization demonstrated bone metastatic disease).

- Renal: serum creatinine < 1.5 x ULN.

9. Exhibit patient compliance and geographic proximity that allow for adequate follow-up.
10. Patient has been informed about the nature of study, and has agreed to participate in the study, and signed the Informed Consent form prior to participation in any study-related activities.
11. No other malignancies within the past five years except adequate treated basal cell or squamous cell skin cancer or carcinoma in situ of the cervix
12. Resolution of all acute toxic effects of prior anti-cancer therapy or surgical procedures to NCICTCAE version 4.0 Grade ≤1 (except alopecia or other toxicities not considered a safety risk for the patient at investigator's discretion).
13. Patient has been informed about the translational sub-study and has agreed to participate in the collection of blood and tumor tissue samples by signing the Informed Consent form.

4.3. Exclusion criteria

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

1. ER or HER2 unknown disease
2. HER2 positive disease based on local laboratory results (performed by immunohistochemistry/FISH)
3. Locally advanced breast cancer candidate for a radical treatment.
4. Prior endocrine therapy in the metastatic setting is not allowed. (Neo)/Adjuvant endocrine therapy is allowed only if the disease-free interval between the end of endocrine therapy and the appearance of metastases in higher than 12 months.
5. Patients with rapidly progressive visceral disease or visceral crisis
6. Have had a major surgery (defined as requiring general anaesthesia) or significant traumatic injury within 4 weeks of start of study drug, patients who have not recovered from the side effects of any major surgery or patients that may require major surgery during the course of the study
7. Patients with an active, bleeding diathesis
8. Have a serious concomitant systemic disorder (e.g. active infection including HIV, or cardiac disease) incompatible with the study (at the discretion of investigator), previous history of bleeding diathesis, or anti-coagulation treatment (The use of low molecular weight heparin is allowed as soon as it is used as prophylaxis intention).

9. Are unable to swallow tablets
10. History of malabsorption syndrome or other condition that would interfere with enteral absorption
11. Chronic daily treatment with corticosteroids with a dose of ≥ 10mg/day methylprednisolone equivalent (excluding inhaled steroids).
12. Known active uncontrolled or symptomatic CNS metastases, carcinomatous meningitis, or leptomeningeal disease as indicated by clinical symptoms, cerebral oedema, and/or progressive growth. Patients with a history of CNS metastases or cord compression are eligible if they have been definitively treated with local therapy (e.g., radiotherapy, stereotactic surgery) and are clinically stable off anticonvulsants and steroids for at least 4 weeks before randomization
13. Known hypersensitivity to letrozole, fulvestrant or any of their excipients, or to any PD-0332991 excipients.
14. QTcF >480 msec on basal assessments, personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP).
15. Uncontrolled electrolyte disorders that can compound the effects of a QTc-prolonging drug (e.g., hypocalcemia, hypokalemia, hypomagnesemia).

5. ASSESSMENTS AND STUDY PROCEDURES

5.1. Patient entry procedures

It should be obtained written patient informed consent prior to undergoing any study related procedures.

Patients will be randomized into the study provided they have satisfied all patient selection criteria.

At the time of randomization, information about patient demographics and stratification factors will be requested. Patients will be stratified based on:

- Site of disease: visceral vs. non-visceral involvement
- Onset of metastatic disease diagnose: Patients with metastasis de novo vs. relapse to prior therapy.

Patients will be assigned to one of the two treatment groups based on a randomisation schedule prepared previously by the sponsor.

Each patient will be identified with a unique patient number (UPN) for this study by the sponsor. All data will be recorded with this identification number on the appropriate CRFs.

5.2. Study assessments

Medical History and Demographic Data: Medical history includes clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal/homeopathic remedies, nutritional supplements) used by the patient within 21 days prior to the screening visit. Demographic data will include age, sex, and self-reported race/ethnicity.

Vital Signs: Vital signs will include measurements of height (at screening only), weight, respiratory rate, heart rate, blood pressure, and temperature. Abnormal or significant changes to vital signs from baseline should be recorded as adverse events, if appropriate.

Physical Examinations: A complete physical examination should include the evaluation of head, eye, ear, nose, and throat; cardiovascular; dermatological; musculoskeletal; respiratory; gastrointestinal; and neurological systems. Changes from baseline abnormalities should be recorded at each subsequent physical examination. New or worsened abnormalities should be recorded as adverse events if appropriate.

As part of tumour assessment, physical examinations should also include the evaluation of the presence and degree of enlarged lymph nodes, hepatomegaly, and splenomegaly. Limited physical examinations will be symptom-directed.

Tumour and Response Evaluations: All patients are evaluable for disease response unless they come off study due any reason other than clinically or radiological confirmed disease progression and have not had any acceptable complete disease assessment.

Measurable and no-measurable disease must be documented at screening and re-assessed at each subsequent tumour evaluation.

Disease assessment should be evaluated preferably by CT or MRI since these methods are the best currently available and reproducible techniques to measure lesions selected for response assessment.

In the event a positron emission tomography (PET)/CT scanner is used for tumor assessments, the CT portion of the PET/CT must meet criteria for diagnostic quality. Tumor assessments should include an evaluation of all known and/or suspected sites of disease, whenever possible. Patients should have lesions selected that can be evaluated at every tumor assessment.

The same radiographic procedure used at screening must be used throughout the study (e.g., the same contrast protocol for CT scans).

If a bone scan cannot be performed during the course of the study because of the unavailability of the Tc-99m isotope, the investigator may choose an alternative imaging modality. See *Appendix 3: Instructions for Scans in the Event of Isotope Shortage*.

Radiographic imaging should always be performed rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical examination. As per RECIST v.1.1 criteria, documentation by colour photography including a ruler to estimate the size of the lesion is recommended.

At baseline all patients should be assessed as follows:

- Assessment of thorax, abdomen, and pelvis
- Bone scan
    - If bone involvement is identified at baseline, bone scan should be repeated every 24 weeks (± 7 days). If bone lesions meet RECIST criteria to be followed by CT, bone lesion can be followed-up as part of the 12-week periodical tumor assessments.

    - If no bone involvement is identified at baseline, it is no necessary to repeat the bone scan unless clinically or biochemically suspected bone progression.

Bone lesions identified at baseline should be followed by bone scans every 24 weeks (± 7 days) regardless that identified bone lesions could contain soft tissue that can be evaluated by CT or MRI.

- CT or MRI of the brain must be obtained at screening only for those patients with clinical suspicion of central involvement. If during the study, patient is diagnosed with CNS metastases, but with disease control outside of the CNS, patient will be allowed to continue receiving study treatment. Medical monitor should be

contacted to agree the best approach to manage patients with CNS involvement on case by case basis.

Tumor response assessments will be performed every 12 weeks (±7 days) from the date of randomization until radiographically and/or clinically documented PD as per RECIST v.1.1, study treatment discontinuation, initiation of new anticancer therapy or discontinuation of patient from overall study participation (e.g., death, patient's request, lost to follow-up), whichever occurs first. Initial tumor response assessment will be performed at the end of cycle 3. Each assessment will be performed as scheduled according to the calendar regardless of any dosing delay to prevent the introduction of bias into the assessment of efficacy. Failure to perform any of the required disease assessments will result in the inability to determine disease status for that time point.

Response assessments will be assessed by the investigator, based on physical examinations, CT or MRI scans, and bone scans using RECIST v. 1.1 (*see Appendix 2: Response Evaluation Criteria in Solid Tumors (RECIST criteria) guidelines (version 1.1)*). At the investigator's discretion, CT scans, MRI scans, and/or bone scans may be obtained at any time when clinically indicated or if progressive disease is suspected.

Laboratory Assessments

Local Laboratory Assessments: Prospective endocrine receptors and HER2 status, haematology and biochemistry. Patients will be enrolled based on local results.

Laboratory test will be performed as per local standard of care and clinical indication before treatment administration. These values should be included: hemoglobin, hematocrit, red blood cell count, platelet count, and white blood cell count with differential count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamm-glutamyl transferase, lactate dehydrogenase, total bilirubin, blood glucose and creatinine.

Electrocardiograms: A 12-lead ECG should be obtained at baseline and be printed and kept with the patient's record. Thereafter, the electrocardiograms will be performed every 3 cycles starting on cycle 3 up to End of treatment visit. The QT interval corrected as per Fridericia's formula must be recorded (QTcF).

ECOG Performance Status: Performance status will be measured using the ECOG performance status scale (see below). It is recommended, where possible, that a patient's performance status be assessed by the same person throughout the study.

Table 1. Scale of ECOG Performance Status

Grade	Scale
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, i.e., 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

(http://www.ecog.org/general/perf_stat.html)

Samples for translational evaluation of molecular biomarkers

Blood samples will be collected from all patients participating in the study at four time points: baseline (prior to first treatment dose), after 2 and 12 weeks of treatment start and at time of progression (before starting new anti-tumor treatment).

Additionally, patients must consent to provide tumor samples from metastatic tissue at baseline and at time of progression (if biopsable lesion at relapse). For non *de novo* patients, tumor tissue at the time of metastatic disease diagnose is requested as preferred option,though at least archived tissue sample from the primary tumor could be also acceptable. If available, archived primary tumor sample must be collected even though tissue sample corresponding to the advanced disease is also obtained prior to study treatment start.

See Appendix 4: Translational evaluation of molecular biomarkers sub study for further details

5.3. Schedule of assessments

Study assessments are outlined in *Appendix 1: Schedule of assessments and study procedures*

Written informed consent for participation in the study must be obtained before performing any study specific screening tests or evaluations. Informed Consent Forms

for enrolled patients and for patients who are not subsequently enrolled (screening failures) will be maintained at the study site.

Results of standard of care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study start may be used (for bone scans that period is up to 60 days prior to the start of study treatment); such tests do not need to be repeated for screening.

All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before study start. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

Visits are based on scheduled 28-day cycles (if no treatment delay due to toxicity occurs). Dose delays and dose reductions will be allowed as outlined in section 6.8. All visits must occur within ± 3 business days from the scheduled date, unless otherwise noted in the schedule of assessments. All assessments will be performed on the day of the specified visit unless a time window is specified. Assessments scheduled on the day of study treatment administration should be performed prior to study treatment administration unless otherwise noted. If the timing of a protocol-mandated procedure falls within a holiday period and/or weekend, it should be performed on the nearest following date (i.e., within 3 business days).

Local laboratory assessments scheduled on Day 1 of all cycles (additional hemograms on D14 of Cycle 1 and 2) must be performed within 72 hours prior to study treatment administration unless otherwise specified. Results of local laboratory assessments must be reviewed and the review documented prior to study treatment administration.

All patients will be closely monitored for safety and tolerability during study treatment and at the follow up period. Patients should be assessed for toxicity prior to any study treatment administration; dosing will occur only if the clinical assessment and local laboratory test values are acceptable.

Efficacy follow up: Initial tumour response assessment will be performed at the end of cycle 3. Thereafter, all patients will be followed up for efficacy every 12 weeks during the study period until progression, patient withdraws consent or death, whichever occurs first. Response and progression will be evaluated in this study using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) (*Appendix 2: Response Evaluation Criteria in Solid Tumors (RECIST criteria) guidelines (version 1.1)*).

Treatment follow up: Patients who discontinue study drugs for any reason will be followed up to EoS (as defined in section 3.7) or up to study termination, whichever occurs first.

The first safety follow up visit for the study treatment will be scheduled for all patients 28 days (+/- 7 days) after the last dose of any IMP.

Afterwards, patients will be followed every 6 months at least, up to EoS or the patient withdraws consent or until death, whichever occurs first. During these follow-up contacts it will be collected survival status and post study anticancer therapy evaluation. Telephone contact is acceptable.

All ≥ grade 2 adverse events will be followed up until improvement to baseline levels, grade 1 or complete recovery, the patient withdraws consent, patient's death or lost to follow-up.

Summary of study assessments will be reported in *Appendix 1: Schedule of assessments and study procedures*

5.4. Patient, study, and site discontinuation

Patient discontinuation: Patients have the right to withdraw from the study at any time for any reason and without having to detail their reasons to do so. The investigator has the right to discontinue a patient from study treatment or from the study for any medical condition that the investigator determines may jeopardize the patient's safety if she continues in the study, for reasons of non-compliance (e.g., missed doses, visits), if the patient becomes pregnant, or if the investigator determines it is in the best interest of the patient.

Patients must be withdrawn from study treatment if they experience disease progression defined using RECIST v. 1.1. The exception to this is patients who develop isolated progression in the brain as described in section 3.4.

Details of discontinuation due to toxicity are given in section 7.

Patients who discontinue from study treatment prematurely will continue to be followed according to section 5.4 with the exception of patients who withdraw consent and have not the willingness to be followed up. The primary reason for discontinuation must be recorded on the appropriate Case Report Form (CRF) section.

Study discontinuation: Section 3.7 describes the EoS definition and discontinuation of study.

A subject will be withdrawn from the study (i.e. from any further study procedure) for any of the following reasons:

- Withdraws consent.
- Lost to follow-up.

In case a subject is lost-to-follow-up, every possible effort must be made by the study site personnel to contact the subject and determine the reason for discontinuation. The measures taken to follow up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented on the CRF and in the source documents. Subjects who withdraw will not be replaced.

Prior to that, the Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients
- Patient enrolment is unsatisfactory
- Steering Committee's recommendation after Interim-analysis results
- Data recording is inaccurate or incomplete

6. STUDY DRUGS INFORMATION

6.1. Drug supplies

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Palbociclib updated information is reported in the current version of the study document *Palbociclib Investigator's Brochure.*

Refer to Investigator's Brochure for fulvestrant (Faslodex®) and summary of product characteristics (SmPC) for letrozole for additional administration instructions and further information.

6.2. Formulation and Packaging

Palbociclib (PD-0332991) will be supplied as capsules containing 75 mg, 100 mg, or 125 mg equivalents of PD-0332991 free base. The sponsor will supply the oral drug

formulation to sites in HDPE (High-density polyethylene) bottles containing 75 mg, 100 mg, or 125 mg capsules. The capsules can be differentiated by their size and colour (see below).

Table 2. Palbociclib Capsule Characteristics

Dosage	Capsule colour	Capsule size
75 mg	Sunset Yellow/Sunset Yellow	2
100 mg	Caramel/Sunset Yellow	1
125 mg	Caramel/Caramel	0

Fulvestrant: will be supplied as two pre-filled syringe (5 ml) that contains each one 250 mg fulvestrant and other ingredients (excipients) like ethanol (96 per cent), benzyl alcohol, benzyl benzoate and castor oil. Fulvestrant is a clear, colourless to yellow, viscous solution in a pre-filled syringe fitted with a tamper-evident closure, containing 5 ml solution for injection. Two syringes must be administered to receive the 500 mg recommended monthly dose.

Letrozole: Commercially available letrozole 2.5 mg film-coated tablets will be used in the study.

6.3. Preparation and Dispensing

Palbociclib will be supplied in non-patient-specific bottles containing either 23 capsules. Fulvestrant will be supplied in non-patient-specific box containing two pre-filled syringe (5 ml).

Commercially available letrozole will be supplied to the patients in non-patient-specific packages containing enough tablets to cover the treatment of the 28-day cycle.

For Palbociclib: The unique patient number (UPN) should be recorded on the box label in the spaces provided at the time of assignment to patient. Site personnel must ensure that patients clearly understand the directions for self-medication. Patients should be given sufficient study medication to cover study treatment 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.

Palbociclib is an agent that must be handled and administered with care. Patients should be instructed to keep their medication in the bottles provided and not transfer it to any

other container. Due to possible unknown hazards associated with topical and environmental exposure to experimental agents, capsules must not be opened and/or emptied into any vehicle for oral ingestion; capsules must be swallowed intact.

Only one bottle containing an specific capsule strength will be dispensed to the patient at each dispensing visit. In the event of dose modification, patient will be requested to return all previously dispensed medication to the clinic before new capsules are dispensed.

For Fulvestrant: The unique patient number (UPN) should be recorded on the box label in the spaces provided at the time of assignment to patient. The administration of this medication should be done at the investigational center/hospital. Refer to the Investigators Brochure for Faslodex® for additional administration instructions.

6.4. Administration

For both palbociclib and letrozole:

- Patients who miss a day's dose entirely must be instructed NOT to "make it up" the next day.
- Patients who vomit any time after taking a dose must be instructed NOT to "make it up," and to resume treatment the next day as prescribed.
- Patients who inadvertently take 1 extra dose during a day must be instructed to skip the next day's dose. Also refer to Section 6.5 for further details on medication errors and overdose.

Palbociclib:

- Patients should take palbociclib capsules with food.
- Patients should be instructed to swallow palbociclib capsules whole and not to chew them prior to swallowing.
- No capsule should be ingested if it is broken, cracked, or otherwise not intact.
- Patients should be encouraged to take their dose at approximately the same time each day.
- Patients should be instructed to record daily administration of the study drugs in the document *Patient Diary*.

Palbociclib will be administered orally once a day for 21 days of every 28-day cycle followed by 7 days off treatment.

Patients experiencing investigational product related toxicity may have their dose modified according to Section 6.8.

Fulvestrant injection should be administered as two consecutive 5 ml injections into the gluteus maximus muscle under pressure (one injection in each buttock). The injection should take approximately 1-2 minutes. The administration of fulvestrant should be done by qualified personnel. It is recommended to remove from the refrigerator for a short period of time until room temperature is reached or rolling the prefilled syringe between the palms of the hands for a short period of time prior to administration. It is important to make sure that the patient is comfortable before the administration. The patient either can lie in a prone or semi-prone position or can stand while leaning forward.

Fulvestrant should be administered every 28 days. During the first cycle it is necessary a loading dose (it will be administered on Days 1, and 14).

Letrozole: Letrozole will be administered orally once daily continuously together with palbociclib or alone in the event of palbociclib dosing interruption.

6.5. 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 appropriate CRF section. In the event of medication dosing error, the sponsor 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 investigational product;
- Potential medication errors or a use outside of what is foreseen in the protocol that does or does not involve the participating patient.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error should be captured on the adverse event (AE) page.

6.6. Compliance

At the beginning of each cycle patients will be required to return all bottles of palbociclib and all packages of letrozole as well as the completed patient diary for drug accountability. The fulvestrant drug accountability will be required to the investigational site staff.

Drug accountability will be performed on Day 1 of every cycle prior to dispensing drug supply for the next cycle. The number of remaining capsules/tablets or the number of injection i.m. administered will be documented and recorded.

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 section 6.8.

6.7. Drug Storage and Drug Accountability

Storage conditions stated in the Study Reference Safety Document (i.e., Investigator's Brochure (IB), Summary of Product Characteristics (SmPC), or Local Product Document (LPD) may be superseded by the label storage.

Investigators and site staff are reminded to continuously monitor room storage temperatures and ensure that thermometers are working correctly as required for proper storage of investigational products. This requirement includes thermometers for both the room storage and refrigerator storage. Any temperature excursions must be reported immediately to the sponsor and documented. Once a deviation is identified, the investigational products MUST be quarantined and not used until the sponsor provides documentation of permission to use the investigational product.

At the end of the trial, the sponsor will provide instructions as to disposition of any unused investigational product. If the sponsor authorizes destruction at the trial site, the investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and any special instructions provided by the sponsor. Destruction must be adequately documented.

Palbociclib: Palbociclib capsules should be stored at controlled room temperature (15-30ºC, 59-86ºF) in their original container.

Medication should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned medication should be stored separately from medication that needs to be dispensed.

To ensure adequate records, palbociclib capsules will be accounted for as instructed by the sponsor. Patients are requested to return previously dispensed containers as well as their completed patient diary to the clinic at each visit for accountability purposes even if they will not be issued with new medication at that visit.

Fulvestrant: Store and transport in a refrigerator (2°C - 8°C).
Temperature excursions outside 2°C - 8°C should be limited. This includes avoiding storage at temperatures exceeding 30°C, and not exceeding a 28 day period where the average storage temperature for the product is below 25°C (but above 2°C - 8°C). After temperature excursions, the product should be returned immediately to the recommended storage conditions (store and transport in a refrigerator 2°C - 8°C). Temperature excursions have a cumulative effect on the product quality and the 28-day time period must not be exceeded over the duration of the 4-year shelf life of Fulvestrant. Exposure to temperatures below 2°C will not damage the product providing it is not stored below – 20°C. Store the pre-filled syringe in the original package in order to protect from light.

Letrozole tablets must be stored according to the instructions detailed in the local package insert.

6.8. Dose Modification

Every effort should be made to administer study treatment on the planned dose and schedule. However, in the event of significant treatment-related toxicity, administration of study drugs (fulvestrant, palbociclib or letrozole) may need to be adjusted as described in the following sections. Depending on the nature of the toxicity observed, dosing adjustment might be required for just one or both study drugs in the combination. In the event treatment interruption is deemed necessary for just one of the study drugs in the combination, treatment with the other study drug will continue as planned.

Dose modifications for letrozole will be aligned to the summary of product characteristics approved locally.

Fulvestrant Dose Modification

No dose adjustment for fulvestrant is permitted but dosing interruptions are allowed.
Treatment interruption for fulvestrant-related toxicities will be performed as per the investigator's best medical judgment. Patients discontinuing fulvestrant treatment

permanently due to treatment-related toxicity will be discontinued from the active treatment phase of the study and enter the follow-up phase.

Palbociclib Dose Modification

In the event of significant treatment-related toxicity, palbociclib dosing may be interrupted or delayed and/or reduced as described below. In the event of multiple toxicities, dose modification should be based on the worst toxicity observed. Patients are to be instructed to notify Investigators at the first occurrence of any adverse sign or symptom.

Dose modifications may occur in three ways:

- Within a cycle: dosing interruption until adequate recovery and dose reduction, if required, during a given treatment cycle;
- Between cycles: next cycle administration may be delayed due to persisting toxicity when a new cycle is due to start;
- In the next cycle: dose reduction may be required in a subsequent cycle based on toxicity experienced in the previous cycle.

Patients discontinuing palbociclib treatment permanently due to treatment-related toxicity may continue on the active treatment phase of the study receiving fulvestrant or letrozole monotherapy as per the investigator's discretion.

Palbociclib: Dosing Interruptions

Management of dose modifications as result of hematologic toxicities is summarised below:

- Grade 1 or 2 : No dose adjustment is required.
- Grade 3 (any haematological toxicity except for lymphopenia unless associated with clinical events, i.e., opportunistic infections).

  On day 1 of cycle: Withhold palbociclib, repeat whole blood monitoring as soon as possible (within a week). When recovered to grade ≤2, start treatment at the same palbociclib dose level.

  On day 14 of initial two cycles: Continue palbociclib treatment at current dose level. Repeat blood count on day 21. Consider dose reduction if prolonged (> 1 week) recovery from grade 3 neutropenia or recurrent grade 3 neutropenia in subsequent cycles.

- Grade 3 ANC + Fever ≥38.5 °C and/or infection: Withhold palbociclib and

initiation of next cycle until recovery to grade ≤2 (≥1.0 x 109/L). Resume at next lower dose.

- Grade 4: Withhold palbociclib and initiation of next cycle until recovery to grade ≤2. Resume at next lower dose.

For non-hematologic toxicities (interstitial lung disease (ILD) and/or pneumonitis not included):

- Grade 1 or 2 : No dose adjustment is required.
- Grade ≥ 3 : withhold palbocicib until recovery to grade <1 (or to grade ≤2 if not considered a safety risk for the patient). Resume at next lower dose.

Appropriate follow up assessments should be done until adequate recovery occurs as assessed by the Investigator.

Doses may be held as needed until toxicity resolution. Depending on when the adverse event resolved, a treatment interruption may lead to the patient missing all subsequent planned doses within that same cycle or even to delay the initiation of the subsequent cycle.

If the adverse event that led to the treatment interruption recovers within the same cycle, then re-dosing in that cycle is allowed. Doses omitted for toxicity are not replaced within the same cycle. The need for a dose reduction at the time of treatment resumption should be based on the criteria defined in following section (Dose Reductions) unless expressly agreed otherwise following discussion between the investigator and the sponsor. If a dose reduction is applied in the same cycle, the patient will need to return to the clinic to receive new drug supply. In the event of a treatment interruption for reasons other than treatment-related toxicity (e.g., non-cancer related surgery) lasting >2 weeks, treatment resumption will be decided in consultation with the sponsor.

If a treatment delay results from decline in hematologic parameters, the frequency of blood count assessments should be increased as clinically indicated.

If these parameters are met within 2 weeks of treatment interruption or cycle delay, palbociclib may be resumed. Please refer to Section Dose Reductions for adverse events requiring dose reduction at the time of treatment resumption.

If these parameters have not been met after 2 weeks of dose interruption (including the scheduled 1 week off treatment) or 2 weeks of cycle delay, permanent discontinuation of palbociclib treatment should be considered. Treatment resumption for patients

recovering from treatment-related toxicity after >2 weeks of treatment interruption or cycle delay but deemed to be deriving obvious clinical benefit per the investigator's best medical judgment is left at the investigator's discretion after sponsor consultation. On a per case basis and after sponsor consultation, in patients with repetitive drug-related toxicities requiring recurrent dose modifications and that are having a clinical benefit, it might be agreed adjustments to the standard dose schedule or to the dose modification guidelines.

In the event that the start of a new cycle is delayed due to treatment related toxicity, all procedures other than tumor assessments scheduled by protocol are required on Day 1 of the given cycle will be performed when palbociclib is resumed (just before palbociclib is restarted) (please note that tumor assessment protocol schedule has to be maintained). New cycle Day 1 procedures (i.e., physical examination, ECOG performance status, ECG, 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.

For ILD and/or pneumonitis:

Severe, life-threatening, or fatal ILD and/or pneumonitis can occur in patients treated with palbociclib when taken in combination with endocrine therapy. Patients must be monitored for pulmonary symptoms indicative of ILD/pneumonitis (e.g. hypoxia, cough, dyspneoa). In patients who have radiological findings suspected of ILD and/or pneumonitis or new or worsening respiratory symptoms and are suspected to have developed ILD/pneumonitis, the administration of palbociclib must be interrupted and patient must be evaluated.

- Grade 1: Palbociclib can be resumed at one dose level lower if the event is fully resolved to Grade 0 within 28 days after last palbociclib dose. In any other case, permanently discontinue subject from palbociclib treatment.
- Grade 2, 3, or 4: Permanently discontinue subject from palbociclib treatment.

Palbociclib: Dose Reductions

Following dose interruption or cycle delay the palbociclib dose may need to be reduced when treatment is resumed. No specific dose adjustments are recommended for Grade

1/2 treatment-related toxicity (with the exception of ILD and/or pneumonitis. See dosing interruptions for ILD/pneumonitis).

However, investigators should always manage their patients according to their medical judgment based on the particular clinical circumstances.

Dose reduction of palbociclib by 1 and, if needed, 2 dose levels (Table 3) will be allowed depending on the type and severity of toxicity encountered. Patients requiring more than 2 dose reductions will be discontinued from the study and entered into the follow-up phase. All dose modifications/adjustments must be clearly documented in the patient's source notes and the CRF.

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

Table 3. Dose reduction of palbociclib

Dose level	Palbociclib for 3 out of 4 weeks (3/1 schedule)
Starting dose	125 mg/d
-1	100 mg/d
-2	75 mg/d
	Discontinue study treatment

QTc prolongation management

In the event of QTc prolongation of, possible alternative reversible causes such as serum electrolytes abnormalities, or usage of concomitant medications with the potential to prolong the QTc interval should be evaluated.

If such reversible causes are identified, then they should be corrected accordingly (i.e., correction of electrolyte abnormalities with supplements to within normal limits and/or discontinuation (if possible) of concomitant medications known to prolong the QT interval).

Recommended dose modifications for palbociclib in the event of QTc prolongation are common to other non-hematological toxicities.

6.9. Potential drug-drug interaction

Palbociclib

In vitro data indicate palbociclib is primarily metabolized by CYP3A4 and sulfotransferase enzyme SULT2A1. Palbociclib is a weak time-dependent inhibitor of CYP3A following daily 125 mg dosing to steady state in humans.

CYP3A Inhibitors: Data from a drug interaction trial in healthy subjects (N=12) indicate that coadministration of multiple 200 mg daily doses of itraconazole with a single 125 mg palbociclib dose increased palbociclib AUCinf and the Cmax by approximately 87% and 34%, respectively, relative to a single 125 mg palbociclib dose given alone.

CYP3A Inducers: Data from a drug interaction trial in healthy subjects (N=14) indicate that coadministration of multiple 600 mg daily doses of rifampin with a single 125 mg palbociclib dose decreased palbociclib AUCinf and the Cmax by 85% and 70%, respectively, relative to a single 125 mg palbociclib dose given alone.

CYP3A Substrates: Palbociclib is a weak time-dependent inhibitor of CYP3A following daily 125 mg dosing to steady state in humans. In a drug interaction trial in healthy subjects (N=26), coadministration of midazolam with multiple doses of palbociclib increased the midazolam AUCinf and the Cmax values by 61% and 37%, respectively, as compared with administration of midazolam alone.

Gastric pH Elevating Medications: Drug-Drug interaction studies show that Concurrent administration of single 125 mg dose of palbociclib with multiple doses of the proton pump inhibitors (PPI) rabeprazole had limited impact on AUCinf (13% decrease). This reduction in overall exposure is not thought to be clinically relevant. Given the reduced effect on gastric pH of H2 receptors antagonists and local antacids compared to PPIs, the effect of dosing these classes of acid-reducing agents palbociclib exposure when given simultaneously with palbociclib free base capsules under fed conditions is expected to be minimal. In another healthy subject study, coadministration of a single dose of palbociclib with multiple doses of the PPI rabeprazole under fasted conditions decreased palbociclib AUCinf and Cmax by 62% and 80%, respectively, when compared with a single dose of palbociclib administered alone.

Effect of Palbociclib on Transporters: In vitro evaluations indicate that palbociclib has low potential to inhibit the activities of drug transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporter (OAT)1, OAT3, organic cation

transporter (OCT)2 and organic anion transporting polypeptide (OATP)1B1, OATP1B3 and bile salt export pump (BSEP) at clinically relevant concentrations.

Effect of Transporters on Palbociclib*:* Based on in vitro data, P-gp and BCRP mediated transport are unlikely to affect the extent of oral absorption of palbociclib at therapeutic doses.

Pharmacokinetic data from study A5481001 indicate that plasma pharmacokinetics of palbociclib are low to moderately variable with generally dose proportional exposures over the dose range evaluated. palbociclib is slowly absorbed and has an elimination half-life of 27 hours. Preliminary results from recently performed phase I study A5481021 (palbociclib in healthy volunteers to estimate the effect of food on the bioavailability of palbociclib) suggest that the administration of palbociclib with food results in more consistent drug uptake and exposure, thus it was concluded that patients should be instructed to take palbociclib with food.

The potential drug-drug interaction (DDI) between palbociclib and letrozole was assessed in the phase I portion of the study A5481003. No potential for drug-drug interaction between both drugs was observed when administered in combination.

Data for DDI between palbociclib and fulvestrant is available from study A5481023. The concurrent administration of fulvestrant did not have a clinically relevant impact on the plasma PK of palbociclib. Likewise, the concurrent administration of palbociclib did not have a clinically relevant impact on the plasma PK of fulvestrant. Therefore, there is no clinically relevant DDI between palbociclib and fulvestrant when the 2 drugs are coadministered.

Patients using drugs known to cause QT prolongation should be monitored closely with serial electrocardiograms.

Fulvestrant

There are no known DDI requiring dose adjustment.

Fulvestrant does not significantly inhibit any of the major Cytochrome P450 (CYP) isoenzymes in vitro, and results from a clinical pharmacokinetic trial involving co-administration of fulvestrant with midazolam also suggest that therapeutic doses of fulvestrant will have no inhibitory effects on CYP3A4.

In addition, available data indicate that there is not clinically relevant change in fulvestrant clearance as result of induction or inhibition of CYP3A4 with rifampicin or

ketoconazole, respectively. Dosage adjustment is not necessary in patients co-prescribed CYP3A4 inhibitors or inducers.

Pregnancy and lactation

As expected with a potent anti-estrogen, studies in animals have shown reproductive toxicity. Fulvestrant is found in rat milk at levels significantly higher than those in rat plasma. The potential risk for humans is unknown. Therefore, use of fulvestrant should be avoided in pregnant or lactating women.

Patients of childbearing potential should use effective contraception during treatment with fulvestrant and for 2 years after the last dose of fulvestrant-containing regimen.

Letrozole

No study relevant DDIs are known for letrozole. Refer to local SmPC for further details.

7. SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

Safety assessments will consist of monitoring and recording protocol-defined AEs, adverse events of special interest (AESIs) and SAEs; measurement of protocol-specified hematology, clinical chemistry, measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug(s).

The Sponsor or its designee is responsible for reporting relevant SAEs to the Competent Authority, other applicable regulatory authorities, and participating investigators, in accordance with International Conference on Harmonization (ICH) guidelines, European Clinical Trials Directive (Directive 2001/20/EC), and/or local regulatory requirements.

The Sponsor or its designee is responsible for reporting unexpected fatal or life-threatening events associated with the use of the study drug to the regulatory agencies and competent authorities by telephone or fax within 7 calendar days after being notified of the event. The Sponsor or its designee will report other relevant SAEs associated with the use of the study medication to the appropriate competent authorities (according to local guidelines), investigators, and central Institutional Review Boards/Ethics Committees (IRBs/ECs) by a written safety report within 15 calendar days of notification.

7.1. Adverse Events Definitions

An adverse event is any untoward medical occurrence in a clinical study patient administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and/or unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, regardless of whether it is considered related to the medicinal (investigational) product. Abnormal test findings should only be reported as an AE if meets any of the following criteria is associated with accompanying symptoms and a general diagnostic term including the symptoms and the abnormal test finding cannot be defined, or requires additional diagnostic testing or medical/surgical intervention, leads to a change in study drug(s) dosing or discontinuation from the study, needs additional concomitant drug treatment or is considered to be an AE by the investigator or by the Sponsor.

The causal relationship between an adverse event and the Investigational Medicinal Product (IMP) will be defined as below:

Not related: The temporal association between the adverse event and the IMP makes a causal relationship unlikely, or the patient's clinical state or the study procedure/conditions provide a sufficient explanation for the adverse event.

Related: The temporal association between the adverse event and the IMP makes a causal relationship possible and the patient's clinical state or the study procedure/conditions do not provide a sufficient explanation for the adverse event.

Each adverse event must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to palbociclib, fulvestrant, letrozole and/or their combination.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-4_QuickReference_5x7.pdf).

The intensity (severity) of an adverse event will be recorded as one of the following:

- Mild - Easily tolerated and does not interfere with normal daily activities, CTCAE Grade 1.

- Moderate - Causes some interference with daily activities, intervention or treatment may be needed. CTCAE Grade 2.
- Severe - Normal daily activities are substantially impaired, hospitalization and/or intervention or treatment is required, CTCAE Grade 3 or 4.
- Fatal - Death, CTCAE Grade 5.
- Not applicable (Clinically significant and asymptomatic laboratory test abnormalities or abnormal assessments, for which no CTCAE grading guidance is applicable but which are considered as AEs).

A mild, moderate or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event. However, a severe event (such as severe headache) may be of relatively minor medical significance and is not necessarily serious. For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Fever of 39 °C that is not considered severe may become serious if it prolongs hospital discharge by a day. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

Adverse Drug Reactions: All noxious and unintended responses to an IMP (i.e. where a causal relationship between an IMP and an adverse event is at least a reasonable possibility), related to any dose should be considered adverse drug reactions. For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function, is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Serious Adverse Events

Per definition, a Serious Adverse Event is defined as any adverse event that either:

- Results in death,
- is life threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person ability to conduct normal life functions),
- is a congenital anomaly/birth defect.

Definition of Life Threatening: An adverse event is life threatening if the patient was at immediate risk of death from the event as it occurred, i.e. does not include a reaction that might have caused death if it had occurred in a more serious form. For instance, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug induced hepatitis can be fatal.

Definition of Hospitalization: Adverse events requiring hospitalization should be considered serious. In general, hospitalization means that the patient has been admitted (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate at the study site. When in doubt as to whether hospitalization occurred or was necessary, the adverse event should be considered as serious.

Social admission, hospitalization for pre-planned treatments or surgical procedures, elective surgery or routine clinical procedures, which are not the result of an adverse event, need not to be considered adverse events. If anything untoward is reported during the procedure, this must be reported as an adverse event and either 'serious' or 'non-serious' attributed according to the usual criteria.

Definition of clinically/medically significant event:
Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Clinically/medically significant events MUST be reported as SAEs.

In this clinical trial and as defined in this protocol, serious adverse events and hospitalizations unequivocally and solely related to established tumor disease progression will NOT be treated as serious adverse events for reporting obligations.

Serious adverse events, if brought to the attention of the Investigator at any time after the cessation of the study treatment and considered by the Investigator to be possibly related to the study treatment (so, in fact serious adverse reactions), will be reported to the Sponsor.

7.2. Adverse Event Reporting

Adverse events will be collected from the signature of informed consent until the safety follow up visit to be done 28 days (+/- 7 days) after the last day of study treatment. All study patients will be carefully monitored for the occurrence of adverse events during this period.

Signs, symptoms and abnormal diagnostic procedure results that are clearly related should be grouped together and reported as a single diagnosis or syndrome whenever possible. Any additional events that fall outside this definition should also be reported separately.

All adverse events must be recorded in the CRF.

Serious Adverse Event Reporting and Timeframe

Reporting requirements will comply with all applicable local regulations for safety reporting.

All protocol defined SAEs and AESIs will be reported to the Sponsor (MedSIR) within 24 hours of when the Investigator or anyone of the site study team becomes aware of it as follows:

- Report all SAEs and AESIs (as defined in this protocol), irrespective of the study drug received by the patient, whether or not this event is considered by the Investigator to be related to study drug, to MedSIR immediately, but in any event no later than 24 hours of any site study team staff becoming aware of the event.
- Full details of the SAE and AESI should be collected and fully documented using the Serious Adverse Event (SAE) form and sent to MedSIR.
- Follow-up information, copies of the results of any tests, the outcome of the event plus the investigator's opinion of IMP relationship to the SAE(s) and AESI(s), and other document when requested and applicable, will accompany the SAE form as available on the day of reporting or provided as soon as possible thereafter.
- The original SAE Report Form and the fax/email confirmation from the sponsor must be kept with the CRF documentation at the study site(s).

All SAE forms will be sent by the investigator or investigator's team to the Sponsor (MedSIR) by email and fax as noted below:

email: ParsifalSAE@medsir.org

fax: + 34 976 20 44 02

SAEs and AESIs will be followed until resolved, a stable outcome is reached, patient is lost to follow-up or dies.

MedSIR will be responsible for ensuring that events are reported within the mandated timeframe to the EMA, and other Competent Authorities, IECs/IRBs and investigator(s), as necessary.

Adverse Events of Special Interest (AESIs)

AESIs must be reported by the Investigator to the Sponsor expeditiously (see section 7.2), regardless of their seriousness (i.e. no more than 24 hours after learning of the event). AESIs for this study include:

- Suspected transmission of an infectious agent by a medication, whereby any organism, virus or infectious particle (e.g. prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. Transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a medicinal product. This term ONLY applies when contamination of a medication is suspected and DOES NOT apply to infections supported by the mode of action, e.g. immunosuppression.
- Potential drug-induced liver injury as assessed by laboratory criteria for Hy's law. The following laboratory abnormalities define potential Hy's law cases and must be reported as an AESI:
    - Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) elevations that are >3 × upper limit of normal (ULN)
    - Concurrent elevation of total bilirubin >2 × ULN (or clinical jaundice if total bilirubin measures are not available), except in patients with documented Gilbert's syndrome. For patients with Gilbert's syndrome, elevation of direct bilirubin >2 × ULN should be used instead.
    -

Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects' research to follow the determinations of the IRBs/IECs. Non-compliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB/IECs.

Report non-compliance immediately, within 24hours to MedSIR, to the study site Principal Investigator and, as necessary, to the IRB/IEC.

Serious Noncompliance

Definition: noncompliance that materially increases risks that result in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

Report serious non-compliance immediately, within 24hours to MedSIR, to the study site Principal Investigator and, as necessary, to the IRB/IEC.

Expedited Reporting to Health Authorities, Investigators, Institutional Review Boards, and Ethics Committees

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following reference documents:

- Palbociclib Investigator's Brochures
- Fulvestrant Investigator's Brochure
- Letrozole, European public assessment report (EPAR)

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

8.1. Sample Size

The study is a randomized, open label, controlled, Phase II clinical trial. The primary endpoint is progression-free survival (PFS) according to RECIST version 1.1. The primary analysis is to compare the efficacy of palbociclib in combination with fulvestrant (interventional arm) versus palbociclib plus letrozole (control arm).

Superiority analysis:

Based on published efficacy data for palbociclib and fulvestrant in similar target population, the investigator hypothesis (H1) is that median PFS in the palbociclib plus fulvestrant arm (31.3 months) will be higher (Hazard Ratio = 0.7) than in the palbociclib plus letrozole group (22 months). Therefore, we will test the null hypothesis (H0) that median PFS survival is equal in both groups.

The analysis will be performed with Log-Rank test. We assumed an exponential survival function. We estimate a 24 months (mo.) accrual period and a 12 mo. treatment period (maximum follow-up of 36 mo.). We planned a randomization (1:1).
Regarding type I and type II errors, we assumed a power of 80% and a two-sided overall alpha error of 5%. An interim analysis will occur at 22 mo. with 89 events (35% of total events expected). The final analysis will be performed at approximately 36 mo. with 254 events and 486 patients included (52% PFS event rate assumed).

According to Lan-DeMets O'brien-Fleming approximation spending function, the two-sided local type I error for testing the null-hypothesis within one interim and final analysis will be 0.001 and, 0.0498, respectively.

Therefore, we should include 243 patients in the control arm and 243 patients in the interventional arm. A total of 486 patients will be included in this study.

The randomization will be stratified. It can be expected that including factors of prognostic importance in the cox regression model as defined for the confirmatory analysis will increase the power as compared to the log-rank test. However, we preferred to take a conservative approach and we accept the sample size calculated without adjusting for prognostic factors.

Non-inferiority analysis:

As per EMEA guidelines, we will switch to non-inferiority analyses if the superiority criteria cannot be met (CPMP/EWP/482/99 EMEA guideline). We will declare non-inferiority if the upper bound 95% confidence interval (95%CI) of HR between median PFS in palbociclib plus letrozole and palbociclib plus fulvestrant arms, will fall within the non-inferiority margin of 1.21. Non-inferiority margin is justified according with the FDA guidance in non-inferiority studies (FDA GUIDANCE: Non-Inferiority Clinical Trials to Establish Effectiveness). They propose to estimate the average effect of the active control over placebo in historical studies and selecting the 95%CI lower bound. Finally, this value should be adjusted to retain at least 50% of the historical effect of active control versus placebo arms. Accordingly the combined effect of PALOMA-1 and 2 studies are (HR: 1.79. 95%CI: 1.47 to 2.18) and the non-inferiority margin is 1.21. With 254 PFS events, if the HR ≤ 0.94 or median PFS in palbociclib plus letrozole is 22 mo. vs. palbociclib plus fulvestrant 23.3 mo. or better, the upper bound 95% of CI will fall within the non-inferiority margin of 1.21, allowing for the determination that both combinations have a similar treatment effect.

The sample size estimation was made using R (package gsDesign), according to the formulas published by Lachin JM and Foulkes MA (1986).(66)

8.2. Analysis Sets

We propose to test efficacy analysis using two analysis sets; the intention-to-treat set, considering all patients randomized regardless of whether they received the randomized treatment, and the "per protocol" analysis set, considering all patients randomized and received the protocol required study drug exposure and processing. Criteria for determining the "per protocol" group assignment would be established by the Steering Committee before the statistical analysis begins. Given our expectation that very few patients will crossover or be lost to follow-up, these analyses should agree very closely. We propose declaring the superiority of interventional therapy, only if shown to be superior using both the "intention to treat" and "per protocol" analysis sets.

We propose to analyses safety profile of treatments using the safety analysis set, considering all patients that receive one drug exposure.

8.3. Primary efficacy endpoint

The primary endpoint is to evaluate the progression-free survival defined as the time from randomization until objective tumor progression or death by any cause. Patients with no progression or death will be censored at the date of their last evaluable imaging. Censoring rules are specified in **Table 5**.

Table 5. Censoring rules for PFS

Situation	Date of progression or censoring	Outcome
Progression documented between scheduled visits	Earliest of: • Date of radiological assessment showing new lesion (if progression is based on new lesion); or • Date of last radiological assessment of measured lesions (if progression is based on increase in sum of measured lesions).	Progressed
Death before first PD assessment	Date of death.	Progressed
Death between adequate assessment visits	Date of death.	Progressed
No progression	Date of last radiological assessment of measured lesions.	Censored

Treatment discontinuation for undocumented progression	Date of last radiological assessment of measured lesions.	Censored
Treatment discontinuation for toxicity or other reason	Date of last radiological assessment of measured lesions.	Censored
Death or progression after more than one missed visit	Date of last radiological assessment of measured lesions.	Censored

PD: Progression disease.

8.4. Primary efficacy analysis

The progression-free survival will be compared between the two groups using a two-sided stratified log-rank test with site of disease (visceral vs. non-visceral) and by the onset of metastatic disease diagnose (de novo metastatic vs. non de novo patients) as strata. We will test the primary endpoint at a nominal levels of 0.001 and 0.0498 at interim and final analysis, respectively.

In the interim analysis after 89 PFS events:

- We will declare that the PFS of palbociclib plus fulvestrant arm is superior from control group if the upper limit of 99,9% confidence interval for the hazard ratio is lower than 1.
- Otherwise, non-inferiority will be declared if the upper limit of 99,9% confidence interval for the hazard ratio is lower than non-inferiority margin of 1.21.

In the final analysis after 254 PFS events:

- We will declare that the PFS of palbociclib plus fulvestrant arm is superior from control group if the upper limit of 95% confidence interval for the hazard ratio is lower than 1.
- Otherwise, non-inferiority will be declared if the upper limit of 95% confidence interval for the hazard ratio is lower than non-inferiority margin of 1.21.

The ITT population will be considered the primary population for superiority analysis. In contrast, per-protocol (PP) population will be the primary population for non-inferiority analysis.

8.5. Secondary efficacy analysis

The intervention arm (palbociclib in combination with fulvestrant) will be compared against the control (palbociclib in combination with letrozole) for all analysis. For timed endpoints (TTP, OS, DoR and TTR), we will use the Kaplan-Meier method and Log-Rank

test followed by multivariable Cox proportional hazards model for adjusting for stratified randomization variables. For binary outcomes (ORR and CBR) we will use chi-squared test for binary outcomes followed by multivariate logistic regression for adjusting for stratified randomization variables. We will examine the residuals to assess model assumptions.

Patients will be stratified by site of disease (visceral vs. non-visceral), and the onset of metastatic disease diagnose (de novo metastatic versus non de novo patients) as strata.

We will calculate Relative Risk (RR) and Hazard ratios (HR) with corresponding 95% confidence intervals to compare dichotomous and time to event variables, respectively. Up-to-date versions of R will be used to conduct analyses. For all tests, we will use two-sided p-values with alpha ≤ 0.05 level of significance.

To assess the impact of potential clustering for patients cared by the same site, we will use mixed cox regression models (see table 6).

Analysis will he performed on the ITT, and per-protocol populations. ITT population will be considered the primary population for the analysis.

8.6. Safety Endpoints

Analysis of safety-related data will be considered at four levels:

- First, the extent of exposure (dose, duration, number of patients) will be examined to determine the degree to which safety can be assessed from the study.
- Second, we will describe and compare clinically relevant test, concomitant medications and adverse events reported in every study group. For adverse events, we will report intensity, causality, body system, action taken, and outcome.
- Third, serious adverse events, deaths and study discontinuations will be described and examined in every study group.
- Finally, patient grade 3 and 4 toxicities in every study group will be classified by MedDRA system organ class and compared between patient baseline characteristics.

The relation between baseline characteristics and severe adverse events (classified in MedDRA SOCs) will be analyzed with chi-squared test followed by multivariate logistic regression with appropriate interaction terms (baseline characteristic × treatment group) (see table 5).

Analysis will he performed on the safety population.

Table 6. Methods of analysis for each variable.

Variable/Outcome	Hypothesis	Outcome measure	Method of analysis
1) Primary efficacy			
Progression free survival (PFS)	Intervention > control arm (ITT population)	RECIST criteria, v1.1 (time to event)	Stratified log-Rank test
	Intervention = control arm (PP population)	RECIST criteria, v1.1 (time to event)	Stratified log-Rank test
2) Secondary efficacy			
Time to progression (TTP)	Intervention > control arm	RECIST criteria, v1.1 (time to event)	Kaplan-Meier survival analysis (Log-Rank test)
Overall response rate (ORR)	Intervention > control arm	RECIST criteria, v1.1 (Binary)	Chi-squared test / Logistic regression
Duration of response (DoR)	Intervention > control arm	RECIST criteria, v1.1 (time to event)	Kaplan-Meier survival analysis (Log-Rank test)
Time to response (TTR)	Intervention > control arm	RECIST criteria, v1.1 (time to event)	Kaplan-Meier survival analysis (Log-Rank test)
Clinical Benefit Rate (CBR)	Intervention > control arm	RECIST criteria, v1.1 (Binary)	Chi-squared test / Logistic regression
Overall survival (OS)	Intervention > control arm	All causes (time to event)	Kaplan-Meier survival analysis (Log-Rank test)
3) Safety			
Adverse events	Intervention ≠ control arm	MedDRA (categorical)	Descriptive methods
Toxicities G3-4	Differences according baseline characteristics	MedDRA (categorical)	Chi-squared / Mann Whitney test/ Logistic regression.
4) Sensitive analysis			
a) Per protocol analysis	Intervention > control arm	All outcomes	Kaplan-Meier survival analysis (Log-Rank test)/ Chi-squared test

b) Adjusting for baseline and stratified randomization factors	Intervention > control arm	PFS/TTP/OS	Multivariable Cox regression models
c) clustering among individuals within a site	Intervention > control arm	PFS	Mixed Cox regression models

8.7. Translational sub-studies

The objective of the statistical analyses of biomarkers is the identification of those markers or combinations of markers which show best association with positive or negative clinical outcome of palbociclib plus fulvestrant or letrozole.

The biomarker analyses will be exploratory. They aim at exploring the potential to predict clinical benefit, by each marker separately and/or by suitable combinations.

Further data on markers will be analyzed, dependent on its availability. According to experience many biomarkers show a skewed statistical distribution across subjects and within subject. Appropriate transformations will be applied to transform these measurements into distributions with an approximate Gaussian shape. These transformations do not change the order of the values, such that non-parametric analyses based on ranks or cut-offs remain unchanged by the transformation. The basic statistics and interdependencies of the different markers will be descriptively investigated.

Markers will be evaluated on a univariate level regarding their potential for prediction (e.g. search or adaptation of cut-offs) of the clinical endpoints. Further multivariate techniques (e.g. Multiple Logistic Regression, Principal Component Analysis with Rotation, Cluster Analysis) will be employed in order to study combinations of markers. Biomarker and Response correlations with clinical covariates will be investigated. It will be checked whether covariates can improve the prediction and whether there is an interaction with the biomarkers. Relevant covariates could become a part of the statistical prediction model. Candidate groupings derived from biomarkers will be checked with time to event variables (e.g. Kaplan-Meier curves, Cox proportional hazard model, log-rank test).

8.8. Missing Data Management

The analysis of the primary and secondary timed endpoints (progression and death) will be based on a log-rank or Cox regression tests and, therefore, not affected by patient withdrawals (as they will be censored) provided that dropping out is unrelated to prognosis. Patients with missing information in other outcomes, such as clinical benefit rate or overall response, will be considered as no responders. Furthermore, we will report reasons for withdrawal for each randomization group and compare the reasons qualitatively.

8.9. Interim analysis

One interim analyses will be performed at 22 months, after 35% of the total PFS events (89 events) have been observed. Interim analysis will evaluate the primary end-point and all safety and efficacy secondary objectives. The trial may be stopped for superiority or non-inferiority at interim according with mentioned decision criteria (refer to 8.1). However, the decision to stop the trial should be agreed by the SC after reviewing the interim safety and efficacy data. The trial may also stop for inferiority if palbociclib plus fulvestrant arm is significantly worse than palbociclib plus letrozole.

8.10. Steering Committee Review

A Steering Committee (SC) has been established for this study. Initially, it is composed of the investigators, the study medical monitor, and the Scientific Global Coordinator. The SC will meet on demand to review, discuss and evaluate all of the gathered safety data. In case of any arising safety concern, these meetings can also be called at any time at request of a participating investigator. At these meetings, MedSIR and the participating investigators must reach a consensus on safety data. MedSIR will prepare minutes from these meetings and circulate them to each investigator for comment prior to finalization.

The study site Investigators and MedSIR will review patient data at least every four months. Each study site Investigator will monitor patients' data for serious toxicities on an on-going basis.

9. ETHICAL CONSIDERATIONS

9.1. Regulatory and Ethics Compliance

The study will be performed and reported in accordance with the guidelines of the International Conference on Harmonization (ICH), and the ethical principles laid down in the Declaration of Helsinki. The study will be also compliance with European Directive 2001/20/EC and any applicable local regulations.

9.2. Institutional Review Board / Independent Ethic Committee:

Conduct of the study must be approved by an appropriately constituted IRB/IEC. Approval is required for the study protocol, protocol amendments, informed consent forms, study subject information sheets, and advertising materials. The IRB/IEC must also be contacted in the event of any major protocol violation.

The investigator, and/or the sponsor when required, must communicate with the IRB/IEC to ensure accurate and timely information is provided at all phases during the study.

The Principal Investigator, and/or the sponsor when required, is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments.

In addition to the requirements to report protocol-defined AEs to the Sponsor, investigators are required to promptly report to their respective IRB/EC all unanticipated problems involving risk to human patients. Some IRBs/ECs may want prompt notification of all SAEs, whereas others require notification only about events that are serious, assessed to be related to study treatment, and are unexpected. Investigators may receive written safety reports or other safety related communications from the Sponsor. Safety reports should be made available to IRB/EC to be reviewed and processed in accordance with regulatory requirements and with the policies and procedures established by their IRB/EC and archived in the site's study file.

9.3. Informed Consent

For each study subject, written informed consent will be obtained prior to any protocol related activities. As part of this procedure, the study site Investigator or designee must

explain orally and in writing the nature, duration, and purpose of the study, and the action of the drugs in such a manner that the study subject is aware of the potential risks, inconveniences, or adverse effects that may occur. The study subject should be informed that she is free to withdraw from the study at any time and with no obligation to specify her reasons. The subject will receive all information that is required by local regulations and ICH guidelines.

The Consent Form must be signed and dated by the patient before her participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

A separate consent form will be signed for patients that participate in the evaluation of molecular biomarkers sub-study This informed consent will be optional to the consent for the main study.

A copy of each signed Consent Form must be provided to the patient.

All signed and dated Consent Forms must remain in each patient's study file and must be available for verification by study monitors at any time.

The Informed Consent Form should be revised whenever there are changes to procedures outlined in the informed consent or when new information becomes available that may affect the willingness of the patient to participate.

For any updated or revised Consent Forms, the case history for each patient shall document the informed consent process and that written informed consent was obtained for the updated/revised Consent Form for continued participation in the study. The final revised IRB/EC-approved Informed Consent Form must be provided to the Sponsor for regulatory purposes.

9.4. Data Protection

The sponsor will ensure the confidentiality of patient's medical information in accordance with all applicable laws and regulations.

The sponsor as Data Controller according to the European Directive on the protection of individuals with regard to the processing of personal data and on the free movement of such data, confirms herewith compliance to Regulation (EU) 2016/679 of the European Parliament and of the Council, of 27 April 2016 in all stages of Data Management.

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, the Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

10. SOURCE DOCUMENTATION, STUDY MONITORING, AND QUALITY ASSURANCE

10.1. Source Data Documentation

Source data refers to all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source documents are original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).

Sponsor's Quality Assurance group may assist in assessing whether electronic records generated from computerized medical record systems used at investigational sites can serve as source documents for the purposes of this protocol.

If a site's computerized medical record system is not adequately validated for the purposes of clinical research (as opposed to general clinical practice), applicable hardcopy source documents must be maintained to ensure that critical protocol data entered into the eCRFs can be verified.

At a minimum, source documentation must be available to substantiate subject identification, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; adequate reporting and follow-up of AEs; administration of concomitant medication; study drug receipt/dispensing/return records; study drug administration information; and date of completion and reason.

Data recorded on the eCRF will be verified by checking the eCRF entries against source documents (i.e., all original records, laboratory reports, medical records) in order to

ensure data completeness and accuracy as required by study protocol. The Investigator and/or site staff must make eCRFs and source documents of subjects enrolled in this study available for review by MedSIR or its representative at the time of each monitoring visit.

The source documents must also be available for inspection, verification, and copying, as required by regulations, officials of the regulatory health authorities (e.g., FDA, EMEA, and others), and/or ECs/IRBs. The Investigator and study site staff must comply with applicable privacy, data protection, and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

The patient must also allow access to the patients' medical records. Each patient should be informed of this requirement prior to the start of the study.

10.2. Study Monitoring and Source Data Verification

Study progress will be monitored by MedSIR or its representative (e.g., a CRO) as frequently as necessary to ensure:
That the rights and well-being of human subjects are protected;

- the reported trial data are accurate, complete, and verifiable from the source documents; and
- the conduct of the trial is in compliance with the current approved protocol/amendment(s), GCP, and applicable regulatory requirements.

Contact details for the team involved in study monitoring will be identified in a hand-out located in the Investigator Site File.

Data recorded on the eCRF will be verified by checking the eCRF entries against source documents (i.e., all original records, laboratory reports, medical records, subject diaries) in order to ensure data completeness and accuracy as required by study protocol. The Investigator and/or site staff must make eCRFs and source documents of subjects enrolled in this study available for inspection by the sponsor or its representative at the time of each monitoring visit.

10.3. Retention of records

Investigators must retain all study records required by the applicable regulations in a secure and safe facility. The Investigator must consult a sponsor representative before

disposal of any study records and must notify the sponsor of any change in the location, disposition, or custody of the study files.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. “Essential documents” are defined as documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. The CHMP requires retention for the maximum period of time permitted by the institution, but not less than 15 years (ICH E6, 4.9.5). It is the responsibility of the sponsor to inform the Investigator/institution as to when these documents no longer need to be retained (ICH E6, 5.5.12).

The study site Investigator must not dispose of any records relevant to this study without either (1) written permission from the Sponsor or (2) providing an opportunity for the Sponsor to collect such records. The study site Investigator shall take responsibility for maintaining adequate and accurate electronic or hard copy source documents of all observations and data generated during this study. Such documentation is subject to inspection by the Sponsor and the FDA and/or EMA (or respective individual EU country regulatory authorities).

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

10.4. Data Quality Assurance

During and/or after completion of the study, quality assurance auditor (s) named by the MedSIR or the regulatory authorities may wish to perform on-site audits. The Investigators are expected to cooperate with any audit and provide assistance and documentation (including source data) as requested.

The Sponsor’s representatives are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the clinical study (e.g., CRFs and other pertinent data) provided that patient confidentiality is respected.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH E6 Good Clinical Practice (GCP) and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's (or designee's) Quality Assurance Department. Inspection of site facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the study conduct and compliance with the protocol, ICH GCP (ICH E6), and applicable country regulatory requirements.

11. DATA MANAGEMENT

11.1. Data Entry and Management

In this study, all data will be entered on to eCRFs in a timely fashion by the Investigator and/or the Investigator's dedicated site staff.

The Investigator must review data recorded in the eCRF to verify their accuracy.

Reconciliation of the data will be performed by the designated CRO. At the conclusion of the study, the occurrence of any protocol violations will be identified and recorded as part of the clinical database. After these actions have been completed and the database has been declared to be complete and accurate, it will be locked and will become available for statistical data analysis.

11.2. Data Clarification

As part of the conduct of the trial, MedSIR may have questions about the data entered by the site, referred to as queries. The monitors and the sponsor are the only parties that can generate a query.

11.3. Data Coding Procedures

Coding of AEs, medical history, and prior and concomitant medications will be performed using standard dictionaries as described in the Data Management Plan.

12. STUDY MANAGEMENT

12.1. Discontinuation of the Study

MedSIR reserves the right to discontinue the study for safety or administrative reasons at any time. Should the study be terminated and/or the site closed for whatever reason, all investigational drugs pertaining to the study must be returned to MedSIR. Any actions required to assess or maintain study subject safety will continue as required, in spite of termination of the study.

12.2. Changes to the Protocol

Any change or addition to this protocol requires a written protocol amendment or administrative letter that must be approved by MedSIR, the Scientific Global Coordinator, the study site Investigator and the IRB/IEC before implementation. This requirement for approval should in no way prevent any immediate action from being taken by the study site Investigator or MedSIR in the interests of preserving the safety of all subjects included in the trial. If an immediate change to the protocol is felt to be necessary by the study site Investigator and is implemented for safety reasons, MedSIR should be notified as soon as possible (within 24 hours if possible) and the IRB/IEC should be informed as necessary.

12.3. Publication Policy Protection of Trade Secrets

All information generated in this study must be considered highly confidential and must not be disclosed to any persons not directly concerned with the study without prior written permission from the Scientific Global Coordinator and MedSIR. However, authorized regulatory officials, the Scientific Global Coordinator or the study site Investigator, and MedSIR personnel (or their representatives) will be allowed full access to inspect and copy the records. All clinical investigational drug, patient bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by Scientific Global Coordinator or the study site Investigator and MedSIR.

The sponsor will ensure that as far as possible results of this study will be published as scientific/clinical papers in high-quality peer-reviewed journals. Preparation of such manuscripts will be made with full collaboration of principal Investigators and in accordance with the current guidelines of Good Publication Practice.

The sponsor must be notified of any intent to publish data collected from the study. Prior approval from sponsor must be obtained before publication of any study related results.

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Appendix 1: Schedule of assessments and study procedures

Study Period	Screening	Treatment period	Treatment follow up period	
Day	**-28 to -1**	**Each cycle (every 28 ± 3 days)**	**28±7 days after last dose treatment**	**Every 6 months[15]**
Informed Consent[1]	X			
ER and HER2 status[2]	X			
Baseline sings/symptoms	x			
Check of inclusion/exclusion criteria	X			
Post-menopausal status confirmation	X			
Medical History[3]	X			
Physical Examination and ECOG status[4,5]	X	X	X	
Weight and Vital signs	X	X	X	
Concomitant Medication Reporting[6]	X	X	X	
Review Patient Diary		X		
AE reporting[7]	X	X	X	
12-lead ECG[8]	X	X	X	
Tumor Assessments[9]	X	X	X	X
Samples for translational sub-study:				
Primary tumor biopsy[10]	X			
Blood samples for translational sub-study[11]	X	X	X	
Biopsies from metastatic lesions[12]	X	X		
Standard Laboratory Procedures:				
Hematology[13]	X	X	X	

Biochemistry[14]		X	X	X	
Treatment Administration					
Arm A:	Fulvestrant 500 mg/5mL (i.m. Injection)		D1, D14 On cycle one and D1 thereafter		
	Palbociclib 125 mg total dose (capsules)		D1 to D21		
Arm B:	Letrozole 2.5 mg total dose (tablets)		D1 to D28 (Continuously)		
	Palbociclib 125 mg total dose (capsules)		D1 to D21		

1. Signed written informed consent obtained prior to any trial-specific procedure.
2. Confirmation of histological diagnosis and specified estrogen, progesterone and HER2 receptors status
3. Complete medical history and demographics (including age, gender, ethnic origin) and all medications taken the last 28 days prior to enrolment will be collected.
4. Physical examination and vital signs (including respiratory rate, blood pressure measurements, heart rate, and body temperature) and weight measurement will be performed prior to enrolment.
5. ECOG performance status will be assessed at screening and before trial drug administration at each cycle.
6. Relevant concomitant medication (including any prescription medication, over-the-counter preparation, or herbal therapy) will be recorded between 21 days preceding first treatment dose until the safety follow up visit (28 +/- 7 days of last IMP dose administration).
7. All AEs occurring during the trial from signature of informed consent until the safety follow up visit (28 +/- 7 days of last IMP dose administration) have to be recorded graded according to NCI-CTCAE, version 4.0.
8. ECG at screening and every 3 cycles starting at cycle 3 and till end of treatment follow-up visit (ECGs should be monitored more frequently, if clinically indicated).
9. Baseline assessments of the, thorax, abdomen and pelvis (preferably CT or MRI in case of contrast allergy) must be performed no more than 28 days before the first trial treatment. Baseline bone scan must be performed no more than 60 days before the first trial treatment Post-baseline assessments are to be performed every 12 weeks +/- 7 days using the same imaging method and where possible obtained at the same institution for an individual patient as used during screening until PD. Bone scans are to be performed every 24 weeks (+/- 7 days) if it has been demonstrated (or if it is clinicaclly or biochemically suspected) bone involvement regardeless if the bone involvement could be identified in the CT scan. CT or MRI of the brain is not mandatory at baseline unless clinical suspicion of central involvement.
10. Primary tumor samples should be collected from non-de novo patients.
11. Blood samples for translational sub studies. collected from all patients . at baseline, after 2 and 12 weeks of treatment start and at tumour progression (prior to start alternative anticancer therapy).
12. Tumor samples must be collected from all patients at baseline and at progression (if biopsable lesion). For non de novo patients if collection of tumor tissue at the time of metastatic disease diagnose is not feasible, at least archived tissue sample from the primary tumor must be provided..
13. Laboratory test will be performed as per local standard of care and clinical indication,. At least hemogram should be performed on D1 and D14 at cycle 1 and cycle 2, and D1 for subsequent cycles.before treatment administration. Parameters assessed should include hemoglobin, hematocrit, red blood cell count, platelet count, and white blood cell count with differential count (neutrophils, lymphocytes, mnocytes, eosinophils, basophils).

14. Laboratory test will be performaed as per local standard of care and clinical indication. At least biochemistry results should be available before treatment administration on D1 of each cycle. Parameters assessed should include sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, alkaline phosphatase, aspartate aminotrnsferase, alanine aminotransferase, gamm-glutamyl transferase, actate dehydrogenase, total bilirubin, blood glucose and creatinine.

15. Treatment follow up period: After discontinuation of study treatment, post -treatment follow-up (including survival status and post study anticancer therapy evaluation) will be collected every 6 months (±7 days) from the last dose of study treatment. Telephone contact is acceptable. If patient discontinued treatment for any reason other than progression, tumor assessment will be included in the assessment.

Appendix 2: Response Evaluation Criteria in Solid Tumors (RECIST criteria) guidelines (version 1.1)

New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). EUROPEAN JOURNAL OF CANCER 45 (2009) 228 – 247

Appendix 3: Instructions for Scans in the Event of Isotope Shortage

Two key suppliers of Tc-99m generators (Chalk River Reactor, Canada and High Flux Reactor, the Netherlands) are expected to close. Supplies from other reactor sources will be unable to meet the expected worldwide patient-care needs. As a result, significant shortages of Tc 99m are expected, and the instructions listed below should be followed:

- Tc-99m bone scans should be obtained as part of the baseline tumor assessment in all patients and should be repeated to confirm a CR or if progression of existing bone lesions and/or the development of new bone lesions is clinically suspected.

If a bone scan cannot be performed at baseline or if the investigator suspects that a bone scan may not be able to be repeated during the course of the study because of the Tc-99m shortage, the investigator may choose F-18 NaF or FDG-PET scan as an alternative.

- If bone lesions are selected as index non-target lesions, they must be apparent on baseline CT scans or other radiographic modalities (e.g., skeletal X-rays that can be repeated in subsequent tumor assessments). Additional scans may be obtained to follow clinically important bone lesions if not visualized on the chest, abdomen, or pelvic CT scan.

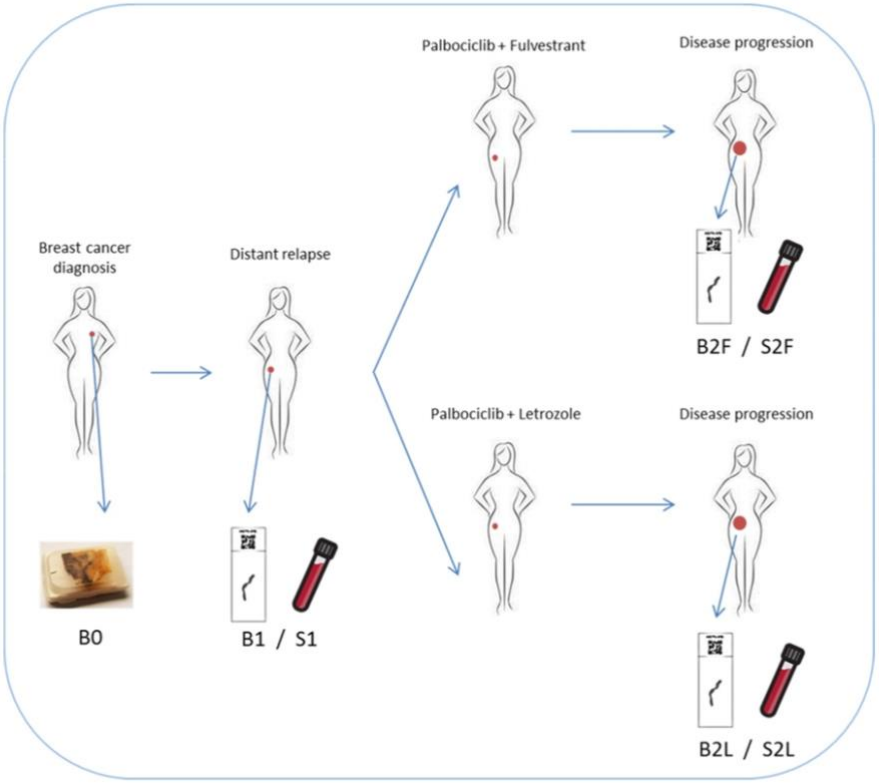
These measures are intended to ensure that the same method of assessment and the same imaging technique is used throughout the study for each patient. If there is a question regarding the choice of alternatives in the event that a standard bone scan cannot be obtained during screening and/or during the study, please contact the Medical Monitor.

Appendix 4: Translational evaluation of molecular biomarkers sub study

The PARSIFAL protocol requires the collection and submission of blood and tumor samples from patients enrolled the study.

Translational sub-studies will be implemented after required regulatory and ethics committee approval..

The blood and tumor samples collected in this study will be used for analysis related for this study as described in the protocol and for studies to be conducted in the future related to the purposes of the Parsifal study but not currently described in the protocol. In addition, as scientific discoveries are made and as science evolves, valuable research can be done in the future on samples collected in the study patients. For this reason, patients would be given the option of consenting for biobanking and use also the samples collected for future research with a broader scope. The procured specimens will not be used for hereditary genetic studies involving genes conferring susceptibility to cancer or other diseases unless additional consent is obtained or an anonymization process is used.



I. Biomaterial

Blood sample collection

At specified timepoints (study entry, after 2 and 12 weeks of treatment start, time to progression) blood samples (up to 2 x 10 ml EDTA/STRECK in whole blood tubes for plasma processing, up to 1 x 10 ml non-EDTA in whole blood tubes for serum processing), are required for consenting patients.

The serum and plasma will be obtained and alliquoted to be stored at -20°C or preferentially at -80 °C. For RNA analysis plasma alliquots preseved in trizol will be used. Futher details on samples preparation and storage will be provided in a separate manual.

All samples will be properly labelled to uniquely identify patient, specimen and time point of collection.

At appropriate time intervals, shimpment of samples to the central storage location will be arranged.

Tumor specimens

Cytological and fine-needle aspiration biopsy samples are not acceptable. The samples must be accompanied by an associated pathology report.

The minimum tissue size is 0.5 x 0.5 x 0.2 cm with ≥10% tumour content or 0.5 x 0.25 x 0.2 cm with ≥20% tumour content or 0.25 x 0.25 x 0.2 cm with ≥ 40% tumour content.

Tumour blocks are preferred, but in the event that local regulations prevent the shipment of tumour blocks, 20 unstained, serially-cut 4-µm-thick slides are requested.
Multiple blocks, or blocks plus slides, can be combined to achieve the minimum requirements indicated.

Futher details on samples preparation and storage will be provided in a separate manual.

The sample collection date, the exact time of collection, and the time of exposure to fixative (formalin) will be properly documented.

A. Biopsies from metastatic lesions

Biopsies from metastatic lesions will be obtained at the time of metastatic disease diagnosis and at the time to progression. Wherever feasible, two samples should be obtained at each time point. The first sample should be placed immediately into 10% buffered formalin. The second biopsy will be placed in sterile tubes, snap frozen in liquid nitrogen or dry ice and stored frozen at ≤ -70°C. Other freezing methods can be

acceptable. If only one sample is available, it should be preserved as a formalin-fixed, paraffin-embedded (FFPE) specimen.

In addition, fresh tissue from patients at defined centres might be obtained for specific analysis (e.g.: patient-derived xenografts studies)

B. Specimens from primary tumor

In case a sample is available, it must be obtained a representative FFPE tumour specimen that with adequate viable tumour cells in a tissue block.

II. Biosample Requirements and Storage

At specific time points, samples will be shipped to the central storage facility (Parc de Recerca Biomedica de Barcelona) where the samples will be logged into the database and assigned a Code Number.

Biomaterials or components extracted from these biomaterials may be transferred to collaborating institutions to perform specific analyses. Samples will not include patient identifiable data and prior to sending any tissue material transfer agreements will be in place with the appropriate parties/collaborators.

III. Description of methods

The multiple assays, described below, may be performed with the material derived from FFPE tumour samples and/or the blood samples collected from each patient as part of this study.

Multiple assays may be performed with the material derived from each patient, but it is likely that not all assays will be performed on samples provided by each patient (possibly because of insufficient tumour material or inadequate sample quality).

All analyses are primarily exploratory in nature. We will employ both a hypothesis-driven and a discovery-based approach.

Analysis to be conducted might include but are not limited to the following:

A. DNA mutation caracterization: sequencing genes in plasma DNA

Mutational analysis in serum and plasma circulating free DNA (cfDNA) will be performed at study entry, during treatment (2 and 12 weeks after treatment start) and at time to progression (or withdraw by toxicity). Results will be used to estimate the amount of tumour-derived cfDNA relative to the total amount of cfDNA for quantitative analyses and case selection for more detailed genetic and epigenetic analyses. Samples with sufficient tumour-derived cfDNA will be subjected to more detailed analysis of mutations or copy number alterations (CNV). Polymerase chain reaction (PCR)-based methods and Massive parallel targeted sequencing (MPS) will be applied to analyze alterations in relevant oncogenes and tumour suppressor genes.

Specifically, the presence of activating Akt1,PIK3CA and ESR1 mutations will be determined. In addition, aberrant methylation of CpG islands of target genes will be performed using sequencing or PCR based techniques.

B. DNA mutation caracterization: sequencing genes in tissue specimens

DNA obtained from FFPE sections of the tissue specimens (primary tumour, diagnosis of metastatic lesion and biopsy with confirmed progression) will be analysed for mutations and copy number alterations. Massive parallel targeted sequencing (MPS) will be applied to determine alterations in relevant oncogenes and tumour suppressor genes using a comprehensive cancer panel (CCP).

Specifically, presence of activating Akt1 or PIK3R1 mutations, inactivating PTEN mutations, ESR1 mutations and alterations in the CCND1 and FGFR1 pathways will be determined. In addition, aberrant methylation of CpG islands of target genes will be performed using sequencing or PCR based methods.

If sufficient DNA remains after the assays above have been performed, genome-wide methods for mutation detection, epigenetic analyses and potentially DNA copy number profiling may be performed. Whole genome-amplified material will be used where necessary. Identified genetic aberrations will be used to anchor subsequent cfDNA analyses.

C. Immunohistochemisry (IHC)

The following antigens will be assayed by IHC: ER, PR, Ki67, cyclin D1, cyclin A1, pRb and others.

Depending on the final number of specimens, tissue microarrays (TMA) may be constructed.

D. Fluorescence In Situ Hybridization (FISH)

HER2, Cyclin D1 and ESR1 gene amplification will be assayed by FISH

E. Exome and RNA sequencing

DNA and RNA will be isolated from snap frozen biopsies according to established protocols. Exome and RNA sequencing will be performed on an Illumina HiSeq Platform. CNV data will be derived from exome sequencing analysis.

In parallel, exosomes will be extracted from plasma samples. Extracted exosomes will subsequently be subjected to RNA, miRNA and protein analyses using appropriate methods including next generation RNA sequencing, PCR-based methods and mass spectrometry.

F. Proteomics: differential protein expression analyses by mass spectrometry

Proteomics has been developed as a complementary approach to the massive sequence of genes and genomes and analysis at the RNA level.

Total protein will be extracted or snap frozen samples.

The identification of early markers of resistance and de novo mechanisms of resistance will require the use of biopsies obtained at relapse (study entry). A comparison of protein expression will be made between patients progressing in the first 3 months vs. the remaining patients. Additionally, findings will be assessed in archival biopsies obtained at the initial diagnosis of breast cancer.

The identification of mechanisms of acquired resistance will require the use of biopsies obtained at initial relapse and at progression. The comparison between these two types of samples will be performed in patients having a progression after 4 months of therapy. Additionally, comparison of samples B2F and B2L would allow the identification of specific mechanisms of resistance depending on the companion hormonal therapy.

The identification of patients who differentially benefit from fulvestrant or letrozole will require the use of biopsies obtained at relapse. The comparison should be established between patients progressing in the first 3-4 months in both treatment groups.

in addition, integrated-genomic biomarkers (iBx) of treatment response will be identified combining drug response parameters and genomic biomarkers based on bioinformatics pipelines designed for PDX data analysis.

G. Clinical trial-matched patient derived xenografts (PDX) models from patients treated with the palbociclib plus fulvestrant/letrozol.

Tumor samples will be implanted into immunosuppressed mice. For those tumor biopsies implanting successfully, the activity of palbociclib plus anti-hormonal therapy (fulvestrant or letrozol) will be tested in vivo as well as using the 3D-ex vivo assay. In vivo studies will assess efficacy of treatment combination and mechansims of cross-resistance. 3D-ex vivo studies would allow to explore treatment combinations that maximize the anti-proliferative activity of palbociclib, including fulvestrant, other SERDs, letrozol and PI3K-pathway inhibitors

in addition, integrated-genomic biomarkers (iBx) of treatment response will be identified combining drug response parameters and genomic biomarkers based on bioinformatics pipelines designed for PDX data analysis.