INTERNATIONAL BREAST CANCER STUDY GROUP IBCSG 53-14 / BIG 14-04

PYTHIA

A Phase II Study of Palbociclib plus Fulvestrant for pretreated patients with ER+/HER2- Metastatic Breast Cancer

Palbociclib in molecularly characterized ER-positive/HER2-negative metastatic breast cancer: the PYTHIA study

EudraCT number: **2014-005387-15**

Pfizer number: **WI198393**

Sponsor: **International Breast Cancer Study Group (IBCSG)**

IBCSG Coordinating Center, Effingerstrasse 40, CH- 3008 Bern
Phone: +41 31 389 93 91 Fax: +41 31 389 93 92

Amendment 1

Protocol Version 2.0

Trial Specific Contacts

Trial Chair	Luca Malorni, MD, PhD USL4 Hospital of Prato, Italy	Email: lmalorni@usl4.toscana.it Tel: +39 (0574) 802523 Fax: +39 (0574) 802903
BIG Headquarters representative	Dimitrios Zardavas, MD	Email: Dimitrios.Zardavas@bigagainstbc.org Tel: +32 2 486 16 64
Chair of PET substudy	Patrick Flamen, MD, PhD Head Dpt. Nuclear Medicine Institut Jules Bordet	Email: patrick.flamen@bordet.be Tel: +32 2 541 3240 Fax: +32 2 541 3224
Trial Statistician	Meredith Regan, ScD	Email: mregan@jimmy.harvard.edu Tel: +1 617-632-3012 Fax: +1 617-632-5444
Trial Data Manager Trial Coordinator	Adriana Mora de Karausch Poonam Jani IBCSG Data Management Center	Email: ibcsg53_PYTHIA@fstrf.org Tel: +1 716-834-0900 (general) Fax: +1 716-833-3990
Translational Research Coordinator	Rosita Kammler	Email: rosita.kammler@ibcsg.org
AURORA Laboratory	AURORA lab OncoDNA s.a. 25 avenue George Lemaître 6041 Gosselies, Belgium	Email: infos@oncodna.com Tel: +32 71 34 78 99 Fax:
Orilab (PET substudy)	Thomas Guiot Camilo Garcia, MD Orilab Institut Jules Bordet	Email: pythia.trial@orilab.org Tel: +32 2 541 3996 Fax: +32 2 541 3224

IBCSG and BIG Contacts

BIG Headquarters	Theodora Goulioti, MD Scientific Director	Email: Theodora.Goulioti@bigagainstbc.org Tel: +32 2 541 7246 Fax:
Coordinating Center	Anita Hiltbrunner Barbara Ruepp, PharmD Manuela Rabaglio, MD Rudolf Maibach, PhD Linda Fritzsche / Sabrina Ribeli-Hofmann Rosita Kammler IBCSG Coordinating Center Effingerstrasse 40 CH-3008 Bern, Switzerland	Email: anita.hiltbrunner@ibcsg.org Email: barbara.ruepp@ibcsg.org Email: manuela.rabaglio@ibcsg.org Email: rudolf.maibach@ibcsg.org Email: drugsupply@ibcsg.org Email: rosita.kammler@ibcsg.org Tel: +41 31 511 94 00 Fax: +41 31 511 94 012

Data Management Center	Lynette Blacher Karolyn Scott Holly Shaw FSTRF 4033 Maple Rd. Amherst, NY 14226 USA	Email: blacher.lynette@fstrf.org Email: scott.karolyn@fstrf.org Email : hshaw@fstrf.org Tel: +1 716 834-0900 Fax: +1 716 833-3990
Data Quality Control Office	IBCSG Data Management Center	Email: ib.dqc@fstrf.org
Center Training Office	IBCSG Data Management Center	E-mail: ibcsg.training@fstrf.org
Statistical Center	Prof. Richard D. Gelber Meredith M. Regan, ScD Dept. of Biostatistics and Computational Biology, CLSB 11007 Dana-Farber Cancer Institute 450 Brookline Ave. Boston, MA 02215 USA	Email: gelber@jimmy.harvard.edu Email: mregan@jimmy.harvard.edu Tel: +1 617-632-3012 Fax: +1 617-632-5444
Scientific Committee Chair	Dr. Marco Colleoni European Institute of Oncology Milan, Italy	Email: marco.colleoni@ieo.it Tel: +39 02 574 89 439 Fax: +39 02 574 89 581

In collaboration with Pfizer Inc.

Protocol Signature Page

IBCSG 53-14

PYTHIA

Approved by:
Group Statistician, International Breast Cancer Study Group
Dr. M.M. Regan

_____ _____

Date

Approved by:
Director, International Breast Cancer Study Group
Anita Hiltbrunner

_____ _____

Date

Approved by:
Associate Scientific Director, Breast International Group
Dr. Dimitrios Zardavas

_____ _____

Date

Approved by:
Trial Chair; Medical Director, U.O. Oncologia Medica "Sandro Pitigliani", Prato ITA
Dr. Luca Malorni

_____ _____

Date

Protocol Signature Page Amendment 1

IBCSG 53-14

PYTHIA

Approved by:
Group Statistician, International Breast Cancer Study Group
Dr. M.M. Regan

30/01/2017

Date

Approved by:
Director, International Breast Cancer Study Group
Anita Hiltbrunner

1.2.2017

Date

Approved by:
Associate Medical, Breast International Group
Dr. Dimitrios Zardavas

30/01/2017

Date

Approved by:
Trial Chair; Medical Director, U.O. Oncologia Medica "Sandro Pitigliani", Prato ITA
Dr. Luca Malorni

30/01/2017

Date

Principal Investigator Protocol Signature Page
Amendment 1

IBCSG 53-14
PYTHIA

I have read the protocol and agree that it contains all necessary details for conducting this trial. I will conduct the trial as outlined in the following protocol and in compliance with GCP, and will apply due diligence to avoid protocol deviations. I will provide copies of the protocol and all drug information relating to pre-clinical and prior clinical experience furnished to me by IBCSG, **to all physicians responsible to me who participate in this trial. I will discuss this material with them to assure that they are fully informed** regarding the drugs and the conduct of the trial. I agree to keep records on all patient information (Case Report Forms and patient's Informed Consent statement), drug-shipment and return forms, and all other information collected during the trial for a minimum period of 15 years.

Name of Principal Investigator:_____

_____ _____

Signature Date

TABLE OF CONTENTS

Section		Page
1.	Protocol Summary and Schema	10
2.	Trial schedule	19
3.	List of abbreviations	21
4.	Background and scientific rationale	23
4.1.	Breast cancer	23
4.2.	Molecular characterization	23
4.3.	CDK inhibition	23
4.4.	Palbociclib	24
4.5.	Fulvestrant	29
4.6.	Biomarkers for palbociclib activity	30
4.7.	Overall risk-benefit assessment	30
4.8.	Rationale for the trial design	31
4.9.	Rationale for the FDG-PET substudy	32
5.	Trial objectives and endpoints	33
5.1.	Primary objective	33
5.2.	Primary endpoint	33
5.3.	Secondary objectives	33
6.	Trial design, duration and termination	34
6.1.	Trial design	34
6.2.	Trial schema	34
6.3.	Treatment	34
6.4.	Clinical evaluations	35
6.5.	Biological evaluations	35
6.6.	Sample size and trial duration	35
7.	Patient selection	35
7.1.	Inclusion criteria	35
7.2.	Exclusion criteria	37
8.	Enrollment	39
8.1.	Patient registration	39
8.2.	Registration Help Desk	39
8.3.	AURORA Registration Confirmation	40
9.	Trial drugs formulation and handling	40
9.1.	Palbociclib	40
9.2	Fulvestrant	41
10	Treatment	41
10.1	Trial treatments	41
10.2	Treatment Administration	42
10.3	Dose modifications and delays for fulvestrant	42
10.4	Dose delays and modifications for palbociclib	43
10.5	Concomitant therapy	45
10.6	Stop of trial treatment	47
10.7	Removal from the trial	48
11	Safety	48
11.1	Adverse reactions to fulvestrant	48
11.2	Adverse reactions to palbociclib	49
11.3	Potential drug induced liver injury	50
11.4	Drug-drug interactions	51

12 Adverse event and serious adverse event reporting 52
12.1 Adverse event reporting 52
12.2 Targeted adverse events 54
12.3 Adverse events of special interest (AESIs) 54
12.4 Otherwise reportable events 54
12.5 Serious adverse event (SAE) 55
12.6 Reporting SAEs 56
12.7 Occupational exposure 57
13 Disease assessment, response and progression (RECIST 1.1) 57
13.1 Introduction 57
13.2 Methods of assessment 57
13.3 Measurability of tumor at baseline 58
13.4 Selection of target lesions 59
13.5 Selection of non-target lesions 59
13.6 Evaluation of target lesions (measurable disease) 60
13.7 Evaluation of non-target lesions 60
13.8 Determination of new lesions 61
13.9 Additional considerations 61
13.10 Determination of time point response 61
13.11 Determination of best overall response 62
13.12 Progression-free Survival 62
13.13 Storage of imaging 63
14 Clinical and laboratory evaluations and follow-up 63
14.1 Screening 63
14.2 Day 1 of every treatment cycle 64
14.3 Treatment day 15 (cycle 1) 64
14.4 Tumor assessments 65
14.5 After discontinuation of trial treatment 65
14.6 Follow-up prior to documented disease progression 65
15 Biological evaluations 66
15.1 Introduction and overview 66
15.2 Translational research 66
16 FDG-PET substudy 67
16.1 Introduction 67
16.2 Study objectives 68
16.3 Study design 68
16.4 Definitions 69
16.5 ORILAB – Imaging CoreLAB: 70
17 Data submission 70
17.1 Case report forms schedule 71
17.2 Signing and submitting forms 72
17.3 Data management 72
17.4 Investigator Site File 72
17.5 Authorization log 74
17.6 Patient identification log 74
18 Statistical considerations 74
18.1 Primary Objective 74
18.2 Secondary Objectives 76
18.3 Definitions of Trial Populations 76
18.4 Interim Analyses 76
18.5 Accrual 76

18.6 Data and Safety Monitoring 77
19 Ethical aspects, regulatory approval, and patient informed consent 77
19.1 Ethical Review Board/Ethics Committee 77
19.2 Regulatory approval procedures 77
19.3 Protection of human patients 77
19.4 Informed Consent 78
19.5 Premature withdrawal 78
20 Governance and Administrative Considerations 79
20.1 Insurance 79
20.2 Steering Committee 79
20.3 Data and Safety Monitoring Committee (DSMC) 79
20.4 Publication of trial results 79
20.5 Premature discontinuation of the trial 80
20.6 Quality Assurance 80
20.7 Protocol adherence 80
20.8 Data protection 80
20.9 Record Retention 81
21 Confidentiality 81
22 References 81

Appendices

1. Requirements for Informed Consent

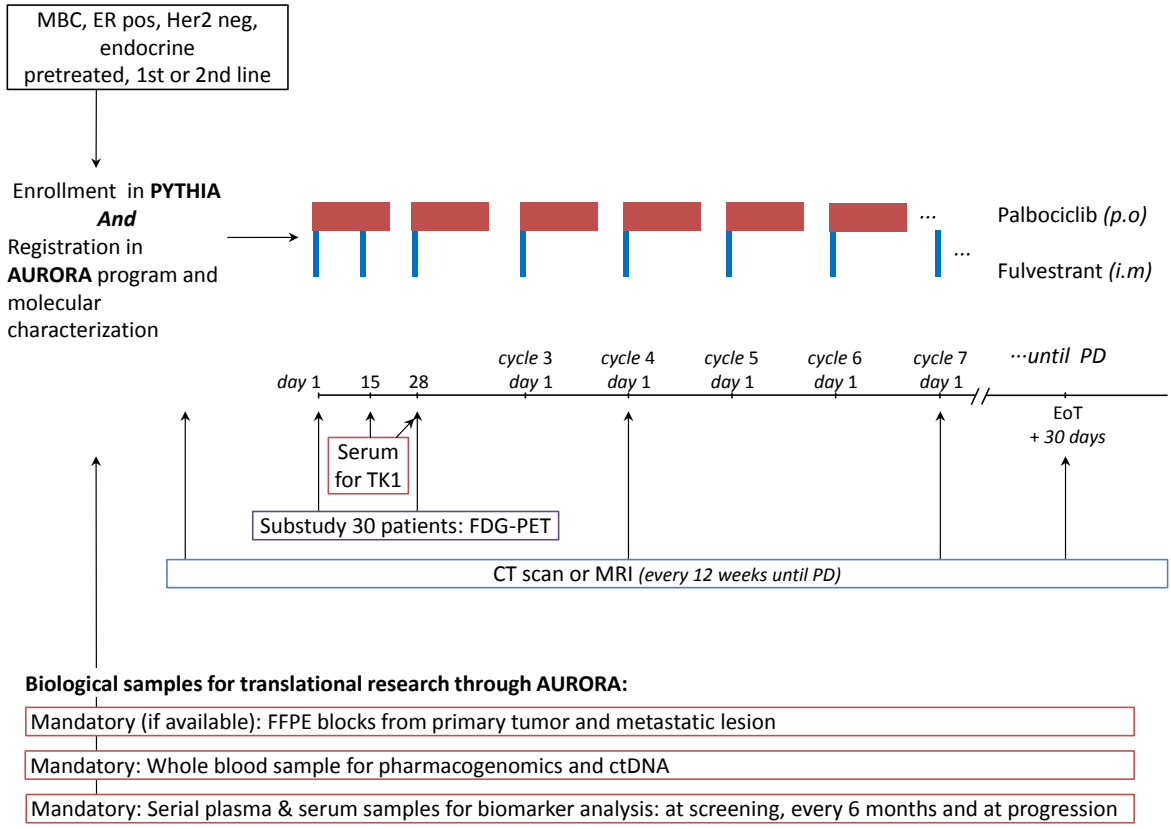
Index of Tables

Table 1. ECOG Performance Status 38
Table 2. NYHA functional classification 38
Table 3. Palbociclib capsule characteristics 41
Table 4. Palbociclib dose reduction/delay for hematological toxicities 43
Table 5. Palbociclib dose reduction/delay for non-hematological toxicities 44
Table 6. Available dose levels 44
Table 7. Adverse reactions based on pooled dataset from 3 randomized studies 49
Table 8. Measurable Disease - Overall Response 61
Table 9. Non-Measurable Disease - Overall Response 62
Table 10. Confidence Intervals (CI) for AE Rates (%) in Experimental Group 76

1. Protocol Summary and Schema

Title	A Phase II Study of Palbociclib plus Fulvestrant for pretreated patients with ER+/HER2- Metastatic Breast Cancer (PYTHIA trial)
Sponsor	International Breast Cancer Study Group (IBCSG)
Pharma Partner	Pfizer
Clinical phase	Phase II
Population	Patients with endocrine-resistant metastatic or locally relapsed, ER+/HER2-negative breast cancer not amenable to treatment with a curative intent enrolled in the AURORA study.
Treatment	Patients will receive: • Palbociclib plus fulvestrant Fulvestrant 500mg will be administered intramuscularly on days 1 and 15 of cycle 1, then on day 1 of every 28 days cycle (+/- 3 days). Palbociclib will be administered orally at a dose of 125 mg per day continuously for 3 weeks followed by 1 week off; repeated at each subsequent cycle of 28 days. Patients will receive the treatment until progression, lack of tolerability, or patient declines further protocol treatment.

Trial Schema



Rationale

This is an international, multicenter, prospective single arm Phase II biomarker discovery clinical trial with the primary objective of assessing the association of PFS with gene mutations, gene copy number aberrations and gene signatures in post-menopausal women with hormone receptor positive, HER2-negative metastatic or locally relapsed breast cancer whose disease has progressed after prior adjuvant endocrine therapy or one line of systemic treatment, i.e., endocrine treatment or chemotherapy, administered for metastatic disease. This trial is included in the AURORA program conducted by the Breast International Group (BIG), an international study aiming to collect and characterize biological samples, including metastatic tissue, from patients with advanced breast cancer. Detailed molecular information (including somatic mutations, copy number variation, gene expression profiling and circulating biomarkers), as well as functional image data for a subset of patients, will be available to identify putative biomarkers of response to the trial regimen.

Palbociclib is a potent, highly selective, reversible, orally active, inhibitor of cyclin dependent kinases 4 and 6 (CDK 4/6). This compound prevents progression of the cell cycle from G1 into the S phase, therefore inhibiting cell growth. Estrogen receptor is a key driver of the proliferation and growth of breast cancer cells and it is a well-known predictive factor for endocrine therapy response. The proliferative program driven by the estrogen receptor requires the transcriptional activation of the Cyclin D1 gene leading to formation of the cyclin D1-CDK4/6-Rb complex, which

	facilitates the G1 to S phase transition. The introduction of cyclin-dependent kinases 4 and 6 (CDK4/6) inhibition in conjunction with endocrine treatment represents an important treatment advancement for patients with hormone receptor (HR) positive, human epidermal growth factor receptor 2 (HER2) negative metastatic breast cancer (O'Leary B, 2016). In particular, when palbociclib was administered in combination with letrozole to treat patients with newly diagnosed metastatic breast cancer in the randomized phase II PALOMA-1 study, progression-free survival (PFS) was significantly improved compared with that in patients treated with letrozole alone (10.2 months (95% CI 5.7-12.6) for the letrozole group and 20.2 months (13.8-27.5) for the palbociclib plus letrozole group, hazard ratio 0.488, 95% Confidence Interval, CI, 0.319-0.748; one-sided p=0.0004) (Finn RS, 2015). Additionally, in the PALOMA-3 randomized phase III trial, the efficacy of fulvestrant plus palbociclib was compared with that of fulvestrant plus placebo in 521 premenopausal and postmenopausal patients with HR-positive, HER2-negative metastatic breast cancer whose disease had relapsed or progressed with previous endocrine therapy. The combination of fulvestrant plus palbociclib was associated with longer PFS than was fulvestrant plus placebo (9.2 versus 3.8 months respectively, hazard ratio for disease progression or death, 0.42; 95% CI, 0.32 to 0.56; P<0.001) (Turner NC, 2015). Abemaciclib, another potent selective CDK4/6 blocking agent, has been recently granted Food and Drug Administration (FDA) breakthrough therapy designation for patients with pretreated HR-positive metastatic breast cancer, on the basis of antitumor activity exerted as monotherapy in a phase I study that reported a clinical benefit rate (CBR) of 61.1% in patients with heavily pretreated metastatic breast cancer (Patnaik A, 2016). The above mentioned results substantiate the notion that the CDK4/6 blockade will become an essential treatment option in the therapeutic armamentarium for patients with advanced luminal breast cancer. Nevertheless, a particular subgroup of patients who are likely to respond better than others to the combination of CDK4/6 blockade with endocrine treatment has not been identified. Indeed, the attempts to the present day to identify predictive biomarkers to guide patient selection for this type of systemic treatment have failed. Preclinical evidence had shown that increased expression of cyclin D1 and phosphorylated Rb and decreased expression of p16 were associated with response to palbociclib (Finn RS, 2009). On the basis of this data, the 165 patients in the PALOMA-1 trial were enrolled into two cohorts, one of which included patients with amplification of cyclin D1 or loss of p16, or both; no predictive relevance of these aberrations was found (Finn RS, 2015). Similarly, attempts to correlate the efficacy of the fulvestrant plus palbociclib combination with either the degree of endocrine sensitivity assessed clinically (i.e., previous endocrine response) or pathologically (i.e., levels of expression of estrogen

	and progesterone receptors, ER and PgR respectively) or to PIK3CA mutational status failed within the context of the PALOMA-3 trial (Cristofanilli M, 2016). Fulvestrant is a potent antiestrogen drug that binds to and induces degradation of ER. Recent data with fulvestrant administered at the dose of 500 mg/month to patients with recurrent hormone receptor positive, HER2-negative breast cancer indicate significant antitumor activity after both antiestrogen and aromatase inhibitor (AI) failures. Fulvestrant is currently indicated for the treatment of postmenopausal women with metastatic hormone receptor positive breast cancer following the failure of antiestrogen therapy. The growing data about the activity of fulvestrant after antihormonal therapy failure together with emerging efficacy data of palbociclib support their combined use in women with hormone receptor positive, HER2-negative metastatic breast cancer whose disease has progressed after prior endocrine therapy. The primary aim of the PYTHIA study is to discover potentially innovative biomarkers for the selection of patients to palbociclib/fulvestrant treatment. The strength of the trial lies in its conduct in conjunction with the AURORA study, which systematically evaluates a panel of biomarkers in tissue and blood, in a certified central lab (Zardavas D, 2014). Stemming from this association, an abundance of molecular profiling information will become available for different biological samples. Additional molecular and functional imaging assessments performed within the context of the PYTHIA study increase its scientific merit, since it will represent a prospective, systematic effort to identify biomarkers for patient stratification, integrating several molecular profiling assessments. References: Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol. 2016 Apr;17(4):425–39 Finn RS, Dering J, Conklin D, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res BCR. 2009;11(5):R77 Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol.

	2015 Jan;16(1):25–35 O'Leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol. 2016 Jul;13(7):417–30 Patnaik A, Rosen LS, Tolaney SM, et al. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors. Cancer Discov. 2016 Jul;6(7):740–53 Turner NC, Ro J, André F, et al. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2015 Jul 16;373(3):209–19 Zardavas D, Maetens M, Irrthum A, et al. The AURORA initiative for metastatic breast cancer. Br J Cancer. 2014 Nov 11;111(10):1881–7
Eligibility	**<u>Inclusion criteria:</u>** • Histologically confirmed breast adenocarcinoma that is metastatic or locally relapsed disease not amenable to curative therapy. • Female gender • Age ≥ 18 years • Postmenopausal, defined as women with: - Prior bilateral surgical oophorectomy; or - Amenorrhea and age ≥ 60 years; or - Age < 60 years and amenorrhea for 12 or more consecutive months in the absence of alternative pathological or physiological cause (including chemotherapy, tamoxifen, toremifene, or ovarian suppression) and FSH and serum estradiol levels within the laboratory's reference ranges for postmenopausal women. • Endocrine resistant disease, defined as one of: - Relapse while on adjuvant endocrine therapy; - Relapse within 12 months after completion of adjuvant endocrine therapy; - Progression of disease under first line endocrine therapy for metastatic and/or loco-regionally advanced breast cancer. Note: Patient may have received one prior chemotherapy for advanced or metastatic breast cancer. • ER positive and HER2-negative tumor, as assessed locally • Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1. • Measurable or non-measurable but evaluable disease according to RECIST 1.1. Bone only disease is allowed. Previously irradiated lesions are deemed measurable only if progression is documented at

	the site after completion of radiation. • Written Informed Consent (IC) for screening procedures and trial participation must be signed and dated by the patient and the Investigator prior to screening. • Written informed consent to participate in the AURORA program of BIG. • The patient has been informed of and agrees to data transfer and handling, in accordance with national data protection guidelines. • Life expectancy >3 months. • Hematological status: - Absolute neutrophil count ≥ 1.5 × 10^9/L (without growth factor support); - Platelet count ≥ 100 × 10^9/L (no transfusion allowed within 2 weeks prior to assessment); - Hemoglobin ≥ 9 g/dL (transfusion permitted). • Hepatic status: - Serum total bilirubin ≤ 1.5 × upper limit of normal (ULN). In the case of known Gilbert's syndrome, a higher serum total bilirubin (< 3 × ULN) is allowed. - AST and ALT ≤ 2.5 × ULN; if the patient has liver metastases, ALT and AST must be ≤ 5 × ULN. • Glucose in normal range, or well-controlled diabetes defined as an HbA1c level ≤ 7.5%. • Renal status: - Creatinine ≤ 1.5 ×ULN or creatinine clearance > 60 ml/min. • International Normalized Ratio (INR) or Prothrombin Time (PT) ≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulant. • Ability to swallow oral medication. **Exclusion criteria:** • Prior use of fulvestrant or any CDK inhibitor. • More than one prior line of chemotherapy for metastatic or locally relapsed disease. • Previous or current non-breast malignancies within the last 5 years, with the exception of in situ carcinoma of the cervix, and adequately treated basal cell or squamous cell carcinoma of the skin. • Known active uncontrolled or symptomatic CNS metastases, carcinomatous meningitis or leptomeningeal disease as indicated by clinical symptoms, cerebral edema, 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,) and are clinically stable off anticonvulsants and steroids

	for at least 4 weeks before enrollment. • Any of the following in the previous 6 months: myocardial infarction, severe/unstable angina pectoris, ongoing cardiac dysrhythmias of NCI CTCAE grade ≥2, atrial fibrillation of any grade, coronary/peripheral artery bypass graft, symptomatic congestive heart failure (NYHA functional classification ≥ III), cerebrovascular accident including transient ischemic attack, or symptomatic pulmonary embolism. • QTc exceeding 480msec, family or personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP). • Uncontrolled electrolyte disorders that can reinforce the QT-prolonging effect of the drug (e.g., hypocalcemia, hypokalemia, hypomagnesemia). • Known history of HIV seropositivity. HIV screening is not required at baseline. • Uncontrolled diabetes defined as HbA1c level > 7.5%. • Concurrent disease or familial, sociological or geographical condition that would make the patient inappropriate for trial participation or any serious medical disorder that would interfere with the patient's safety. • Dementia, altered mental status, or any psychiatric condition that would prevent the understanding or rendering of Informed Consent. • Known abnormalities in coagulation such as bleeding diathesis, or treatment with anticoagulants precluding intramuscular injections of fulvestrant. • Treatment with an investigational agent in the 4 weeks before enrollment. • Concurrent treatment with any of the drugs not permitted, i.e., strong CYP3A inhibitors/inducers and drugs known to cause QT interval prolongation. • Adverse events (except alopecia) from previous systemic cancer therapy, radiotherapy or surgery have not recovered to CTCAE v4.0 grade 1 or resolved prior to enrollment.
Trial Objectives and Endpoints	**<u>Primary objective</u>** The primary objective of this single-arm phase II trial is to interrogate in a prospective manner a series of different molecular aberrations, assessed at a central lab, as well as functional imaging data both at baseline and after one cycle of treatment for a subset of 30 patients; all those potential biomarkers will be assessed for their associations with the progression-free survival of patients receiving fulvestrant plus palbociclib treatment and they can be listed as follows: a. Gene mutations in a panel of genes assessed on ctDNA, as determined by the AURORA platform

	b. Gene mutations and gene copy number aberrations of an extended panel of cancer-related genes measured on primary tumors and/or tissue from metastatic biopsies, as determined by the AURORA platform. c. Baseline levels of activity of TK1 as well as early changes in TK1 levels measured during and after one cycle of therapy. d. Gene signatures as inferred by RNA sequencing that will be performed through the AURORA program. In particular, in addition to other gene signatures measuring the activational status of several oncogenic signaling pathways, the following gene signatures with potential predictive utility in terms of sensitivity to CDK4/6 blockade will be assessed, namely: i. A gene signature called 'RBsig' consisting of 87 genes that is indicative of loss-of-function of the Retinoblastoma (Rb) pathway. ii. A gene signature developed at Université libre de Bruxelles consisting of 11 genes that is indicative of the phosphorylation status of CDK4. **Secondary objectives** To evaluate: • Safety and tolerability, as documented according to NCI CTCAE v4.0. • Disease control, based on RECIST 1.1 criteria.
FDG-PET substudy	An FDG-PET substudy will be conducted on a subset of 30 patients from selected sites. While the substudy is too small to draw definitive conclusions, FDG-PET early response may be a promising tool particularly for clinical development of new drugs or combinations.
Translational research	From the AURORA program, detailed molecular information (including somatic mutations, copy number variation, gene expression profiling and circulating biomarkers) will be available. In particular, the following four types of biospecimens will be collected and analyzed: • Archived primary tumor samples • Metastatic tumor tissue • A plasma sample at baseline • A whole blood sample at baseline There will be a targeted gene sequencing for all biospecimens perfomed using a panel of 411 cancer related genes for the tissue samples and a panel of 27 cancer related genes for the plasma sample. For successful inclusion in the AURORA program, the successful sequencing of at least 2 out of these 3 biospecimens is required.

<table>
<tr><td></td><td>These will be correlated to the endpoints of the present trial to identify putative biomarkers of response to the trial regimen.

In the AURORA program, a whole blood and a plasma sample for pharmacogenomics will be taken at the time of inclusion of the patient; plasma and serum samples will be taken every 6 months and at time of disease progression to monitor the disease and to better understand the contribution of ctDNA to the evolution of the disease.

Translational research proposals not outlined in this protocol will be assessed by a trial-specific research committee for merit and feasibility.</td></tr>
<tr><td>Statistical considerations</td><td>The primary objective of this single arm phase II trial is to assess the association of the primary endpoint progression-free survival (PFS) with biomolecular markers. 120 patients will be included and treated with palbociclib plus fulvestrant.

The median PFS of patients treated with palbociclib plus fulvestrant is expected to be in the range of 9 to 10.5 months, with about 67% of patients having documented PFS events (~80 events) at the time of analysis. For sample size and power considerations we take the simplified situation wherein PFS is compared between groups of patients with and without a certain biomarker. The table below shows the detectable hazard ratios with 80% power for alternative scenarios in which all 120 patients have biomarker data or conservatively in which ~80% (N=90) patients have biomarker data; and on the basis of nominal two-sided α=0.05 level test and α=0.001 level test as a rough consideration of multiple testing. The sample size of 120 was selected to have 80% power to detect a HR of 2.0 for biomarker with 30-50% prevalence (two-sided α=0.05).
<table>
<tr><td rowspan="3">Analyzable Sample Size</td><td>2-sided α-level</td><td colspan="6">Biomarker Prevalence</td></tr>
<tr><td></td><td>5%</td><td>10%</td><td>15%</td><td>20%</td><td>30%</td><td>50%</td></tr>
<tr><td></td><td colspan="6">Detectable HR</td></tr>
<tr><td rowspan="2">N=120</td><td>0.05</td><td>4.2</td><td>2.85</td><td>2.4</td><td>2.2</td><td>2.0</td><td>1.9</td></tr>
<tr><td>0.001</td><td>8.3</td><td>4.65</td><td>3.65</td><td>3.2</td><td>2.75</td><td>2.5</td></tr>
<tr><td rowspan="2">N=90</td><td>0.05</td><td>5.25</td><td>3.35</td><td>2.75</td><td>2.5</td><td>2.2</td><td>2.1</td></tr>
<tr><td>0.001</td><td>>10</td><td>5.9</td><td>4.5</td><td>3.8</td><td>3.2</td><td>2.9</td></tr>
</table></td></tr>
<tr><td>Number of patients</td><td>This trial will enroll 120 patients.</td></tr>
<tr><td>Duration of trial</td><td>Enrollment is expected to be completed within 12 months. The final trial analysis is expected 30-36 months after the inclusion of the first patient under the amendment. The end of the trial is defined as last patient last visit and is expected to be 36 months after enrollment of the first patient under amendment 1.</td></tr>
</table>

2. Trial schedule

(See section 14 for detailed examinations schedule)

	≤28 days prior to enroll-ment	Day 1 of every 28 days cycle [2]	Day 15 of cycle 1	Weeks 12, 24 etc (every 12 weeks)	Within 30 days after end of treatment [7]	Follow-up every 12 weeks until PD [15]	at pro-gression
Informed consent [1]	x						
Medical history	x						
Physical examination, ECOG PS, height, weight [3]	x	x	x [3]		x	x	
Post-menopausal status [4]	x						
Baseline symptoms and adverse events [5]	x						
Adverse events [5]		x	x		x		
Concomitant medications	continuously from 28 days prior to enrollment until 28 days after treatment stop						
Treatment							
Fulvestrant		x	x				
Palbociclib		x [6]					
Laboratory tests							
Complete blood count [8]	x	x	√		x		
Biochemistry [9]	x	x			x		
Coagulation profile [10]	x	√	√		√		
Tumor Evaluation							
Clinical [11]	x			x		x	x
CT or MRI [11]	x			x	x [13]	x	x
Bone scan [11]	√			√		√	√
FDG PET [12]	√			√		√	√
Cardiac evaluation [14]							
Electrocardiogram	x	√			x		
Research assay							
Serum/plasma sample [16]	(x)	x	x				(x)
Registration into AURORA	x						

x = mandatory √ = if medically indicated

Legend for trial schedule:

1. Informed consent may be obtained earlier than within 28 days before enrollment.
2. Cycle visits should take place every 28 days, +/- 3 days
3. Physical examination should be performed according to local standards. Height needs to be recorded only at baseline. Day 15 of cycle 1: physical examination only.
4. Postmenopausal, defined as
   - Prior bilateral surgical oophorectomy; or
   - Amenorrhea and age ≥ 60 years; or
   - Age < 60 years and amenorrhea for 12 or more consecutive months in the absence of alternative pathological or physiological cause (including chemotherapy, tamoxifen, toremifene, or ovarian suppression) and FSH and serum estradiol levels within the laboratory's reference ranges for postmenopausal women
5. Baseline symptoms and adverse events should be recorded from signature of informed consent to prior to start of treatment. Adverse events occurred during the previous cycle should be checked at

day 1 visit of next cycle, at end of treatment visit and up to progression. Serious adverse events will be collected from day of signature of informed consent until 28 days following the end of treatment.

Treatment

6. Dispense palbociclib to patient for next 3 weeks of this cycle. In case of toxicity, refer to section 10.4 for guidance on delaying or reducing the dose of palbociclib. Collect and check patient diary of previous cycle; hand out patient diary for next cycle.
7. End-of-treatment visit within 30 days after last dose, or at the time of decision to stop the trial treatment if the decision is taken >30 days after last dose

Laboratory tests

8. Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count). To be repeated on day 1 of every treatment cycle prior to start of treatment, if not done within 3 days prior to this day.
9. Biochemistry: serum potassium, HbA1c, glucose, sodium, calcium, LDH, ;

   Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;

   Kidney function tests: urea, creatinine.

   To be repeated on day 1 prior to start of treatment if not done within 3 days prior to this day
10. Coagulation function: prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR).

Tumor evaluation

11. Clinical and radiological (by CT scan or MRI) tumor assessments will be performed at baseline (preferentially within 28 days, but at the maximum 42 days before enrollment), then every 12 weeks (+/- 2 weeks) from enrollment until progression. Bone scan will be performed if clinically indicated at the same time points.
12. Per RECIST criteria, FDG-PET/CT is not foreseen for regular response assessments (see section 13.2). The FDG-PET substudy will be done on a subgroup of patients from selected sites with an FDG-PET done at baseline, **maximum 2 weeks prior to start of treatment**, and after cycle 1, on day 25-28.
13. To be performed if not done already within 30 days prior to this visit.

Cardiac evaluation

14. Electrocardiogram will be done at baseline, thereafter if medically indicated, and again at end of treatment visit.

Follow-up schedule

15. Follow-up visits should be scheduled every 12 +/- 2 weeks at same time as imaging for tumor evaluation and documentation of any anti-tumor therapy between EoT and tumor progression. in case trial treatment was stopped prior to objective disease progression, follow-up continues until progression is documented, or for a maximum of 12 months after treatment stop

Research assay

16. Two serum samples for TK1 assay should be taken on day 15 (+/- 2 days) of cycle 1 and between day 26 of cycle 1 and before any cycle 2 trial treatment, at the latest on day 35. Serum samples at baseline and at progression and marked as "(x)" are taken through the AURORA program, see note below.

    A plasma sample for ctDNA must be taken at baseline (= AURORA screening sample)

Note on biological studies (see section 15)

As part of AURORA, two formalin-fixed, paraffin-embedded (FFPE) tumor blocks, one from the original diagnosis of the primary breast cancer and one from the diagnostic core or excisional biopsy of a loco-regional or metastatic lesion will be available in the AURORA biobank.

As part of AURORA, serum and plasma samples for biomarker analyses will be available in the AURORA biobank. They will be collected at screening into AURORA and every 6 months thereafter, as well as at time of tumor progression.

3. List of abbreviations

AE	Adverse event
AESI	Adverse event of special interest
AI	Aromatase Inhibitor
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANC	Absolute Neutrophil Count
AST	Aspartate transaminase
BIG	Breast International Group
CDK	Cyclin Dependent Kinase
CI	Confidence interval
CNS	Central Nervous System
ctDNA	Circulating tumor DNA
CR	Complete Response
CRF	Case Report Form
CT	Computed tomography
CTCAE	Common toxicity criteria for adverse events
CYP3A	Cytochrom P450 3A4
DC	Disease control
DDPLS	Dedifferentiated Liposarcomas
DLT	Dose-limiting toxicity
DMC	Data Management Center
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EEA	European Economic Area
EORTC	European Organization for Research and Treatment of Cancer
EoT	End of Treatment
ER	Estrogen receptor
ERB	Ethical Review Board
ESR1	Estrogen receptor 1
FDG	Fluoro-D-Glucose
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence in situ hybridization
FLT	Fluorothymidine
FSH	Follicle-Stimulating Hormone
FSTRF	Frontier Science and Technology Research Foundation
GBM	Glioblastoma
GCP	Good clinical practice
GGT	Gamma-Glutamyl Transferase
HbA1c	Glycated haemoglobin
HDPE	High Density Polyethylene
HER2	human epidermal growth factor receptor 2
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IB	Investigator's Brochure
IBCSG	International Breast Cancer Study Group
IC	Informed consent
IC50	Half Maximal Inhibitory Concentration
ICH	International Conference on Harmonization

iDF	iDataFax
IHC	Immunohistochemistry
INR	International Normalized Ratio
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
LD	Lethal Dose
MBC	Metastatic breast cancer
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
mTOR	Mechanistic Target of Rapamycin
NCI	National Cancer Institute
NE	Not Evaluable
NIMP	Non-Investigational Medicinal Product
NYHA	New York Heart Association
OHRP	Office for Human Research Protection
ORR	Overall response rate
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression free survival
PgR	Progesterone receptor
PI	Principal Investigator
PIS/IC	Patient information sheet / informed consent
PR	Partial response
pRb	Phosphorylated Retinoblastoma
PS	Performance status
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QD	Once daily (Quaque Die)
QTc	Corrected QT Interval
QTcF	Corrected QT Interval using the Fridericia correction
Rb	Retinoblastoma
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended phase II dose
SAE	Serious adverse event
SCID	Severe Combined Immunodeficiency
SD	Stable disease
shRNA	Small hairpin RNA
SPC	Summary of Product Characteristics
SPIM	Standard Procedures Imaging Manual
SUSAR	Suspected Unexpected Serious Adverse Reaction
SUV(max)	Maximum Standardized Uptake Value
TEV	Tumor Evaluation Form
TdP	Torsade de Pointes
ULN	Upper limit of normal

4. Background and scientific rationale

4.1. Breast cancer

Breast cancer represents a major health concern worldwide, with approximately 1,000,000 newly diagnosed cases each year[1]. To the present day, therapeutic decisions are being taken on the basis of the expression status of three biomarkers, namely: estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor (HER2). Extensive efforts have been made to molecularly characterize this common disease, with several molecular subtypes being identified[2-6]. An extensive repertoire of pathogenic molecular aberrations driving tumor cell growth have been identified[7-11], with the hope being that therapeutic targeting of these aberrations will improve the clinical outcome of patients with breast cancer[12].

4.2. Molecular characterization

Despite this molecular heterogeneity governing breast cancer, dysregulation of normal cell cycle control seems to be a ubiquitous feature to many different subtypes[13]. Under normal conditions the cell cycle is a tightly regulated step-wise process, consisting of distinct phases. One important step is the transition from G1 to S phase, regulated by the interaction between the cyclin dependent kinases (CDKs) and the cyclin proteins, with the former being serine/threonine kinases[14]. Shortly, CDK4 and CDK6 mediate hyperphosphorylation of the retinoblastoma (Rb) gene product in early G1. This results in Rb inactivation, with the consecutive release of transcription factors inducing progression to the S phase.

4.3. CDK inhibition

Accumulating preclinical evidence supports CDK inhibition as a promising therapeutic strategy for human breast cancer. Indeed, a wide number of molecular aberrations affecting CDKs and their corresponding cyclins have been identified. Cyclin D1 (CCND1) gene amplification and overexpression of the encoded cyclin D1 protein have been found in up to 50% of breast cancer cases[15] and cyclin E over-expression correlates strongly with poor clinical outcome across all subtypes of early stage breast cancer[16], luminal breast cancer[17] and a cohort of untreated patients with HER2-overexpressing disease[18]. Additionally, dysregulation of the network of CDKs and cyclins has been shown to mediate trastuzumab resistance in HER2-overexpressing breast cancer cells and tumour xenograft models, through cyclin E overexpression[19].

Importantly, strong preclinical evidence supports that CDKs and cyclin network dysregulations mediate endocrine resistance: high expression of cyclin E2 (CCNE2) was strongly predictive of shorter distant metastasis-free survival following endocrine therapy[20]. In the same study expression of either cyclin E1 or E2 in T-47D breast cancer cells conferred acute antiestrogen resistance, suggesting that cyclin E overexpression contributes to the antiestrogen resistance of tamoxifen-resistant cells. Moreover, a different study *in vitro* showed that PD0332991 (palbociclib), a selective CDK4/6 inhibitor, shows increased antitumor activity preferentially in luminal ER positive human breast cancer cell lines[21]. In this study, palbociclib showed synergy with tamoxifen in ER-positive breast cancer cell lines.

Despite this striking clinical activity, the identification of predictive biomarkers of sensitivity and/or resistance to CDK inhibition for luminal breast cancer patients is of paramount importance. To this end, solid understanding of the cell cycle regulation by the CDKs-cyclins' network and results generated by preclinical research can be valuable. In the study showing the preferential *in vitro* activity of palbociclib against luminal ER positive human breast cancer cell lines, potential biomarkers were identified: Rb and cyclin D1 were elevated and CDKN2A (p16) was decreased in the most sensitive cell lines[21]. Another study showed that chronic loss of Rb was specifically associated with emergence of a CDK4/6 independent state and ultimately resistance to palbociclib in a panel of breast cancer cell lines[22]. Loss of Rb has been associated with resistance to CDK inhibition in the setting of other cancer cell types, thus indicating that it could serve as a major biomarker of resistance.

Loss of Rb function can be mediated by inactivating mutations that have been reported, albeit at low frequencies (0.4% and 3% for luminal A and B breast cancer cases respectively)[8]. However, additional mechanisms have been reported in human breast cancer resulting in Rb functional inactivation, namely: loss of heterozygosity at the RB1 gene locus[23], overexpression of cyclin D1 and/or amplification of the cyclin D1 gene (CCND1)[15] and gene silencing of the CDK-inhibitor p16ink4a contributing to the deregulation of Rb phosphorylation[24]. A way to capture the functional status of the Rb pathway is through gene expression profiling. Indeed, a gene signature of Rb loss consisting of 159 genes has been reported to be a negative prognostic factor in ER-positive breast cancer[25]. Such a gene signature could be of predictive value for patient selection that will respond to CDK inhibition.

From the above mentioned information, it becomes clear that detailed molecular characterization of tumor samples will be of paramount importance to identify the molecular subgroup of luminal breast cancer patients that will derive benefit from the CDK inhibition therapeutic strategy. To this end, AURORA, the most recent research initiative of Breast International Group (B.I.G.) focusing on metastatic breast cancer offers a unique opportunity: By applying next generation sequencing for a panel of 411 cancer-related genes to blood, as well as both primary tumors and their matched metastatic lesions, AURORA will generate an abundance of molecular profiling information that can be mined to identify potential predictive biomarkers associated with sensitivity and/or resistance to the CDK4/6 inhibition plus fulvestrant treatment utilized in the present study

4.4. Palbociclib

4.4.1. In vitro data

Palbociclib is a highly selective inhibitor of CDK4/cyclinD1 kinase activity (IC50 = 11 nM; K_i = 2 nM). Palbociclib is selective for CDK4/6, with little or no activity against a large panel of 274 other protein kinases including other CDKs and a wide variety of tyrosine and serine/threonine kinases. CDK6, another enzyme that also complexes with cyclin-D subunits, is also commonly expressed in mammalian cells and tumors. CDK6 is highly homologous to CDK4 and can perform the same function by phosphorylating Rb, thus potentially creating a redundant mechanism to promote cell cycle progression. Consequently, inhibition of both enzymes is necessary to ensure complete suppression of Rb phosphorylation and the greatest

possible spectrum of antitumor activity. Results indicate that palbociclib inhibits CDK6 with equivalent potency to CDK4.

The only known natural substrate for CDK4/cyclinD1 is the retinoblastoma gene product, Rb. Specific CDK4 phosphorylation sites on Rb include serine-780 and serine-795. Therefore, the phosphorylation status of Rb at these specific sites in treated tumors can serve as an appropriate biomarker for target modulation by palbociclib. The IC50 for reduction of Rb phosphorylation at serine-780 in the MDA-MB-435 breast carcinoma cell line was 0.066 µM. Palbociclib was equally effective at reducing Rb phosphorylation at serine-795 in this tumor cell line with an IC50 of 0.063 µM. Similar effects on serine-780 and serine-795 phosphorylation were obtained in the Colo-205 colon carcinoma cell line.

Palbociclib inhibits cellular proliferation and prevents cellular DNA synthesis by preventing cells from entering S phase of the cell cycle. Palbociclib inhibited thymidine incorporation into the DNA of a panel of Rb-positive human breast, colon, and lung carcinomas, with IC50 values ranging from 0.040 to 0.17 µM. Palbociclib was also effective in preventing cell cycle progression in human leukemias and in nontransformed human epithelial cells and fibroblasts and was equally effective in suppressing cell division in human tumor cell lines.

A selective CDK4/cyclinD inhibitor should cause a specific accumulation of cells in G1, but have no effect on other phases of the cell cycle, in which cells should continue to progress and eventually decline in number. MDA-MB-453 breast carcinoma cells that were exposed to various concentrations of palbociclib for 24 hours show a significant increase in the percentage of cells in G1 in the presence of as little as 0.04 µM palbociclib with a concomitant decline in other phases of the cell cycle. Finally, to provide further evidence of the selectivity of palbociclib, the compound was tested against Rb-negative tumor cells, which should not be sensitive to a specific CDK4 inhibitor. Palbociclib was tested against the MDA-MB-468 human breast carcinoma and the H2009 human non-small cell lung carcinoma, both of which have deleted Rb. The compound had no anti-proliferative activity on these cells when assayed at 3 µM (highest concentration tested), which is 1 to 2 orders of magnitude higher than the concentration necessary to inhibit Rb-positive tumor cells.

Published reports demonstrate palbociclib produced a potent G1 cell cycle arrest and induced senescence in a panel of 16 Rb-positive glioblastoma (GBM) cell lines, whereas 5 Rb-negative cell lines were resistant[26]. shRNA knockdown of Rb expression conferred resistance of GBM cells to palbociclib. Palbociclib was effective against intracranial GBM xenograft tumors, including those that had recurred after temozolomide therapy. Palbociclib combined favorably with radiation against GBM xenografts[26].

4.4.2. In vivo data

A comprehensive survey of molecular alterations was conducted in 207 patient samples representing seven major subtypes of soft tissue sarcoma[27]. Among prominent alterations discovered were mutations in PIK3CA, TP53, and NF1, along with a complex pattern of amplification of chromosome 12q in 90% of dedifferentiated liposarcomas (DDPLS). The most strongly amplified gene among these 12q amplifications in DDLPS was CDK4. shRNA knockdown of CDK4 inhibited proliferation of two DDLPS cell lines, LPS141 and DDLS8817, as did treatment with palbociclib.

The MTD in SCID mice was 150 mg/kg/day when administered orally, once a day, for 14 days. The MTD was defined as the highest dose that was nonlethal (<LD10). At the MTD on this regimen, palbociclib has significant antitumor efficacy against multiple human tumor xenograft models. The Colo-205 model is exquisitely sensitive to palbociclib. At doses as low as 12.5 mg/kg, a 13-day growth delay was obtained, indicating a 90% inhibition of tumor growth rate. Palbociclib was inactive against the H23 lung and the SW-620 colon carcinomas. The lack of response may be associated with the presence of oncogenic K-ras mutations in SW-620 and H23; none of the xenografts sensitive to palbociclib had such mutations.

Further evidence that the anti-tumor activity observed in Rb-positive tumors is due to inhibition of CDK4/CDK6 protein kinase activity was obtained by testing palbociclib in the MDA-MB-468 breast carcinoma and the DU-145 prostate tumor models. These are Rb-negative tumors; neither of which responded to this compound. The lack of efficacy in Rb-negative tumors is consistent with the lack of anti-proliferative activity observed *in vitro*. Taken together, these results support the proposed mechanism of palbociclib (inhibition of CDK4/6-mediated Rb phosphorylation) and the specificity of the compound demonstrated in enzyme activity tests.

Further studies investigated whether continuous daily dosing of palbociclib was needed for optimal efficacy. Four dosing schedules were employed against the MDA-MB-435 breast carcinoma model over 14 days of treatment, including continuous daily, every other day, every third day, and 3 courses of 3 days dosing followed by 4-day drug holidays. The design of this experiment was such that the total compound administered over the 2-week period was identical for each treatment schedule. The results show that a similar degree of efficacy was attained with all schedules, implying that an intermittent regimen is feasible without compromising activity. Similar experiments were conducted against the Colo-205 colon carcinoma model. Again, intermittent schedules were as efficacious as daily dosing, with tumor regressions occurring during all dosing regimens.

During the 14-day treatment period employed for most of the efficacy experiments, no cures were documented, and the tumors grew back after therapy. It is possible that a tumor variant had selectively grown back and acquired resistance to the compound. To address this possibility, Colo-205 colon tumors that had initially significantly regressed in response to treatment with palbociclib were harvested and reimplanted into naive mice. After the tumors grew to 100 to 150 mg, these tumor-bearing mice were treated with palbociclib with a dose and dosing schedule identical to the original experiment. The tumors responded with equal sensitivity to the drug and fully regressed, indicating that no resistance had developed during the initial treatment. A similar result was observed with retreated MDA-MB-435 tumors.

4.4.3. Prior phase I/II/III non-breast cancer trials

The first in-human study of palbociclib was conducted among 33 patients with Rb-positive advanced solid tumors or non-Hodgkin's lymphoma refractory to standard therapy, exploring a 2 weeks on, 1 week off schedule with once daily administration (QD)[28]. Six patients had dose-limiting toxicities (DLT) (18%; four receiving 200mg QD; two receiving 225mg QD); the maximum tolerated dose (MTD) was 200mg QD. Treatment-related, non-hematological adverse events occurred in 29 patients (88%) during cycle 1 and in 27 patients (82%)

thereafter. Adverse events were generally mild to moderate. In terms of antitumor activity, there were 31 patients out of the total 33 that completed at least one post-treatment tumor assessment and were thus considered evaluable for response[28]. One patient with non-seminomatous testicular cancer, previously treated with bleomycin, cisplatin and etoposide in the adjuvant setting, had a PR with palbociclib administered at 200 mg QD on Schedule 2/1. An additional nine patients (29%) experienced stable disease (SD) lasting ≥2 cycles. Of note, no patient with breast cancer was included in this first in-human trial.

Another phase I study was conducted among 41 patients with Rb-positive advanced tumors to identify the DLT and MTD of palbociclib in six dose-escalation cohorts following a standard 3 + 3 design[29]. DLTs were observed in five patients (12%) overall; at the 75, 125, and 150 mg once daily dose levels. The MTD and recommended phase II dose of palbociclib was 125 mg once daily. Neutropenia was the only dose-limiting effect. After cycle 1, grade 3 neutropenia, anemia, and leukopenia occurred in five (12%), three (7%), and one (2%) patient(s), respectively. The most common non-hematologic adverse events included fatigue, nausea, and diarrhea. In terms of antitumor activity, none of the 37 patients evaluable for response met RECIST guidelines for partial response [29]. However, 13 patients (35%) maintained SD for at least 2 cycles, with one cycle corresponding to a period of 3 weeks on followed by 1 week off, one among whom had breast cancer. SD lasted ≥4 cycles in 10 patients (27.0%) and ≥10 cycles in 6 patients (16.2%). Regarding the latter, there was one patient with breast cancer receiving 50 mg once daily, with high levels (>80%) of Rb-positive cells.

Subsequent studies focusing on specific cancer diagnoses have been performed and reported. A phase I pharmacodynamic study of palbociclib was conducted in 17 patients with relapsed mantle cell lymphoma, using 2-deoxy-2-[(18)F] fluoro-D-glucose (FDG) and 3-deoxy-3[(18)F] fluorothymidine (FLT) positron emission tomography (PET) to study tumor metabolism and proliferation, respectively, in concert with pre- and on-treatment lymph node biopsies to assess Rb phosphorylation and markers of proliferation and apoptosis[30]. The patients received 125 mg of palbociclib monotherapy QD, 3 weeks on, 1 week off. Substantial reductions in the summed FLT-PET maximal standard uptake value [SUV(max)], as well as in Rb phosphorylation and Ki-67 expression, occurred after 3 weeks in most patients, with significant correlations among these end points. Concerning antitumor activity, 5 patients achieved PFS time exceeding 1 year (range, 14.9-30.1+ months), with 1 CR and 2 PRs (18% objective response rate; 90% confidence interval, 5%-40%). These patients demonstrated > 70%, > 90%, and ≥ 87.5% reductions in summed FLT SUV(max) and expression of pRb and Ki67, respectively, parameters necessary but not sufficient for long-term disease control.

A phase II monotherapy trial has been conducted in molecularly selected patients with advanced liposarcoma[31]. A total of 30 patients with Rb-positive disease at the IHC level and CDK4 amplified as assessed by fluorescence in situ hybridization (FISH) received 200mg palbociclib QD for 14 consecutive days in 3-week cycles; 12-week PFS was the primary endpoint. Promising antitumor activity was noted, with 12-week PFS reaching 66% (90% CI, 51% to 100%), median PFS being 18 weeks and one case of PR at 74 weeks[31]. In terms of toxicities, grade 3 to 4 events included anemia (17%), thrombocytopenia (30%), neutropenia

(50%), and febrile neutropenia (3%). Dose reduction for hematologic toxicity was required for 24% of patients.

4.4.4. Prior trials in metastatic breast cancer

In the setting of metastatic breast cancer, the final results of PALOMA-1 (also known as Study 1003 and TRIO-18), a randomized phase II trial, were recently reported [32]. This study evaluated the palbociclib 125 mg QD for 3 weeks followed by 1 week off plus Letrozole combination as 1st line treatment for post-menopausal patients with ER-positive/HER2-negative metastatic breast cancer as compared to Letrozole monotherapy. The study consisted of two parts: Part 1 enrolled patients with no further biomarker assessment besides ER+/HER2– biomarkers while Part 2 enrolled patients additionally screened for CCND1 gene amplification and/or loss of p16. The primary endpoint was investigator-assessed PFS defined as time from randomization to objective progression or death. Secondary endpoints included OR, OS, safety, and correlative biomarker studies. A total of 165 patients were randomized in PALOMA-1; 66 patients in Part 1 and 99 patients in Part 2. Baseline characteristics were balanced between treatment arms. The final analysis of primary endpoint showed a statistically significant improvement in PFS for the investigational arm (20.2 months) vs. the Letrozole arm (10.2 months) with hazard ratio (HR)=0.488 (95% CI: 0.319, 0.748) and 1-sided p=0.0004[32]. The treatment effects were also demonstrated when Part 1 and Part 2 were analyzed separately (HR=0.299 [95% CI: 0.156, 0.572]; 1-sided p=0.0001 for Part 1 and HR=0.508 [95% CI: 0.303, 0.853]; 1-sided p=0.0046 for Part 2). The OS analysis with 61 events demonstrated a trend favoring the palbociclib plus Letrozole combination vs. Letrozole (37.5 months vs. 33.3 months, respectively; HR=0.813; p=0.2105). In terms of toxicities observed, the most common adverse events in the palbociclib plus Letrozole arm were neutropenia, leukopenia, fatigue, and anemia, consistent with the toxicity profile of palbociclib previously reported. Intriguingly, the benefit derived from the palbociclib and letrozole combination was mainly driven by the population not having a CCND1 gene amplification and/or loss of p16 (HR for the biomarker-positive and biomarker-negative patient population was 0.37 and 0.19, respectively, for the comparison of palbociclib plus letrozole versus letrozole arms)[32].

These data were recently confirmed in a larger, double-blind, randomized, phase III registration trial with the same design of PALOMA-1, the PALOMA-2 trial. In this trial, 666 postmenopausal women with ER-positive, HER2-negative BC, who had not received prior treatment for advanced disease, were randomized in a 2:1 fashion to receive palbociclib plus letrozole or placebo plus letrozole. The primary endpoint was PFS, as assessed by the Investigators. Results showed that the median progression-free survival was 24.8 months (95% confidence interval [CI], 22.1 to not estimable) in the palbociclib plus letrozole group, as compared with 14.5 months (95% CI, 12.9 to 17.1) in the placebo plus letrozole group (HR, 0.58; 95% CI, 0.46 to 0.72; p<0.001). Subgroup analyses did not reveal any group of patients selectively receiving benefit from the combination. Adverse events in PALOMA-2 confirmed the overall very favourable toxicity profile of palbociclib seen in the PALOMA-1 trial. The most common grade 3 or 4 adverse events were neutropenia (occurring in 66.4% of the patients in the palbociclib plus letrozole group vs. 1.4% in the placebo plus letrozole group), leukopenia (24.8% vs. 0%), anemia (5.4% vs. 1.8%), and fatigue (1.8% vs. 0.5%). Febrile

neutropenia was reported in 1.8% of patients in the palbociclib plus letrozole group and in none of the patients in the placebo plus letrozole group. Permanent discontinuation of any trial treatment as a result of adverse events occurred in 43 patients (9.7%) in the palbociclib plus letrozole group and in 13 patients (5.9%) in the placebo plus letrozole group [33].

In the setting of endocrine pre-treated metastatic breast cancer, results from the phase III PALOMA-3 trial have recently been published [34, 35]. This trial involved patients with ER-positive/HER2-negative metastatic breast cancer that had relapsed or progressed during prior endocrine therapy. Patients were randomized in a 2:1 ratio favoring the combination arm, to receive palbociclib and fulvestrant or placebo and fulvestrant. Premenopausal or perimenopausal women were eligible and also received goserelin. Patients who had received one line of prior chemotherapy for the treatment of metastatic disease were eligible for this trial. A total of 521 patients were randomized in this study, 347 in the palbociclib and fulvestrant arm and 174 in the palbociclib and placebo arm. The majority of the patients had a post-menopausal status (79.3% in both arms) and received one or more prior lines of therapy for metastatic disease (patients who received no prior treatment for metastatic disease represented 24.2% and 25.9% of the trial population in the palbociclib+fulvestrant and the placebo+fulvestrant arms, respectively). The combination of fulvestrant plus palbociclib was associated with longer PFS than was fulvestrant plus placebo (9.2 versus 3.8 months respectively, hazard ratio for disease progression or death, 0.42; 95% CI, 0.32 to 0.56; P<0.001)[36] . Toxicities were mostly in line with previous data. The rate of discontinuation due to adverse events was 2.6% with palbociclib and 1.7% with placebo. Overall survival data are still immature and require longer follow up.

A detailed quality of life analysis in patients participating in the PALOMA-3 study was recently published. This analysis showed that patients randomized in the palbociclib plus fulvestrant group reported a significant improvement in the global quality of life scores. Also, a greater improvement in pain from baseline was observed in this group with a significantly delayed deterioration in global quality of life and pain compared with fulvestrant alone [37].

4.4.5. Prior Pharmacokinetic Studies

In the aforementioned first in-human phase I trial, additional pharmacokinetic experiments were performed: Palbociclib was slowly absorbed (mean Tmax 4.2h) and eliminated (mean half-life time 26.7h). Volume of distribution was large (mean 3241 L) with dose-proportional exposure[28]. In the second phase I dose-finding study, similar pharmacokinetic characteristics were observed: Palbociclib was slowly absorbed (median Tmax was found to be 5.5 hours), and slowly eliminated (mean half-life was 25.9 hours) with a large volume of distribution (mean, 2,793 L)[29]. The area under the concentration–time curve increased linearly with dose.

4.5. Fulvestrant

Fulvestrant is a potent anti-estrogen drug that binds to and induces degradation of ER. Recent data with fulvestrant administered at the dose of 500 mg/monthly to patients with recurrent hormone receptor positive, HER2-negative breast cancer, indicate significant antitumor activity after both antiestrogen and aromatase inhibitor (AI) failures[38]. Fulvestrant is currently indicated and remains the standard of care for the treatment of postmenopausal

women with metastatic hormone receptor positive breast cancer following the failure of antiestrogen therapy.

4.6. Biomarkers for palbociclib activity

Currently, there is a lack of biomarkers to identify patients more likely to achieve a response to CDK4/6 inhibitors. Efforts have been made towards the identifications of single biomarkers in the CyclinD1/CDK4-6/RB1 pathway itself or in other co-activating pathways such as the PI3K pathway, but have proven unsuccessful so far. This suggests that single biomarkers may not capture the complex biology of resistance to CDK4/6 inhibitors and that a more thorough evaluation of the pathway activity may be necessary.

Genetic loss of RB1 is a marker of primary resistance to CDK4/6 inhibitors but it is uncommon in HR+ or HER2+ subtypes. Recent studies have shown that functional loss of the CyclinD1/CDK4-6/Rb pathway can be measured by gene-expression studies. Several gene-signatures of RB-loss have been developed which have shown to be prognostic in breast cancer subtypes as well as predictive of response to neoadjuvant chemotherapy [39, 40]

The above mentioned results substantiate the notion that the CDK4/6 blockade will become an essential treatment option in the therapeutic armamentarium for patients with advanced luminal breast cancer. Nevertheless, a particular subgroup of patients who are likely to respond better than others to the combination of CDK4/6 blockade with endocrine treatment has not been identified. Indeed, the attempts to the present day to identify predictive biomarkers to guide patient selection for this type of systemic treatment have failed. Preclinical evidence had shown that increased expression of cyclin D1 and phosphorylated Rb and decreased expression of p16 were associated with response to palbociclib [21]. On the basis of this data, the 165 patients in the PALOMA-1 trial were enrolled into two cohorts, one of which included patients with amplification of cyclin D1 or loss of p16, or both; no predictive relevance of these aberrations was found [32]. Similarly, attempts to correlate the efficacy of the fulvestrant plus palbociclib combination with either the degree of endocrine sensitivity assessed clinically (i.e., previous endocrine response) or pathologically (i.e., levels of expression of estrogen and progesterone receptors, ER and PgR respectively) or to PIK3CA mutational status failed within the context of the PALOMA-3 trial [35].

We have recently developed a gene signature of functional loss of Rb (RBsig) that is prognostic in luminal breast cancer subtypes and can predict response to the CDK4/6 inhibitor palbociclib in cell line models of breast cancer [41]. In particular, cell lines with increased levels of RBsig (RBsig HIGH) are among the most resistant to palbociclib treatment.

4.7. Overall risk-benefit assessment

The metastatic or advanced setting was chosen to evaluate this new therapy combination. The setting of incurable disease is acceptable to justify the evaluation of a potential new combination in breast cancer. As these patients are resistant to previous endocrine therapy, an investigational therapy that could potentially reverse this resistance is of high interest in this patient population. The available data provide scientific rationale for the combination of palbociclib and fulvestrant in this patient population. The tolerability of the combination in this population has been shown to be acceptable in the PALOMA-3 trial. Safety will be carefully monitored throughout the trial.

4.8. Rationale for the trial design

This is an international, multicenter, phase II clinical trial for post-menopausal women with hormone receptor positive, HER2-negative metastatic or locally relapsed breast cancer whose disease has progressed after prior endocrine therapy (1st or 2nd line). Patients will be treated with the combination of palbociclib and fulvestrant. The primary objective is to assess the association of the primary endpoint progression-free survival (PFS) with potential markers. This trial is included in the AURORA program, an international study aiming to collect and characterize biological samples, including metastatic tissue, from patients with advanced breast cancer. Therefore, for patients participating in the current trial, detailed molecular information (including somatic mutations, copy number variation, gene expression profiling and circulating biomarkers) will be available for retrospective studies aiming to identify putative biomarkers of response to the study regimen.

Palbociclib is a potent, highly selective, reversible, orally active, inhibitor of cyclin dependent kinases 4 and 6 (CDK 4/6). This compound prevents progression of the cell cycle from G1 into the S phase, therefore inhibiting cell growth. ER is a key driver of the proliferation and growth of breast cancer cells and it is a well-known predictive factor for endocrine therapy response. The proliferative program driven by the ER requires the transcriptional activation of the cyclin D1 gene leading to formation of the cyclin D1-CDK4/6-Rb complex which facilitates the G1 to S phase transition.

The introduction of cyclin-dependent kinases 4 and 6 (CDK4/6) inhibition in conjunction with endocrine treatment represents an important treatment advancement for patients with hormone receptor (HR) positive, human epidermal growth factor receptor 2 (HER2) negative metastatic breast cancer [32, 36, 42]. Abemaciclib, another potent selective CDK4/6 blocking agent, has been recently granted Food and Drug Administration (FDA) breakthrough therapy designation for patients with pretreated HR-positive metastatic breast cancer, on the basis of antitumor activity exerted as monotherapy in a phase I study that reported a clinical benefit rate (CBR) of 61.1% in patients with heavily pretreated metastatic breast cancer [43].

The primary aim of the PYTHIA study is to discover potentially innovative biomarkers for the selection of patients to Palbociclib/Fulvestrant treatment. The strength of the trial lies in its conduct in conjunction with the AURORA study, which systematically evaluates a panel of biomarkers in tissue and blood, in a certified central lab [44]. Stemming from this association, an abundance of molecular profiling information will become available for different biological samples. Additional molecular and functional imaging assessments performed within the context of the PYTHIA study increase its scientific merit, since it will represent a prospective, systematic effort to identify biomarkers for patient stratification, integrating several molecular profiling assessments.

At present, there is lack of data regarding predictive biomarkers to palbociclib in the clinical setting. Pre-clinical data suggest that aberrations in genes and proteins related to the CDK4-6/Cyclin D1 pathway might be predictive of response to palbociclib, but clinical validation has failed so far to confirm these hypotheses analyzing primary tumor tissue samples. A major obstacle to the development of predictive biomarkers for metastatic breast cancer patients is the unavailability for molecular studies of tissue samples from the metastatic site where it is possible to capture the current biology of the disease. The present trial will provide an

important addition to the current knowledge in the field by providing extensive molecular characterization of the metastatic disease and of circulating biomarkers that might be better suited for retrospective biologic correlative studies.

In its meeting of November 7, 2016, the Data and Safety Monitoring Committee (DSMC) for this trial discussed the currently available information about the safety and efficacy of the combination of palbociclib and fulvestrant in this patient population. The DSMC concluded that a randomization between palbociclib plus fulvestrant vs. placebo plus fulvestrant was not justified anymore, and that all patients should receive the active drug. Study Partners, i.e. IBCSG and BIG, have therefore decided to convert the trial into a single arm phase II.

4.9. Rationale for the FDG-PET substudy

Up to the present day, there has been a shortage of data regarding the evaluation of the clinical utility of FDG-PET (fluorodeoxyglucose positron emission tomography) for patients with advanced breast cancer, which is even more pronounced in the setting of treatment with targeted agents. There have been small studies with populations of less than 20 patients that reported promising results with regards to the predictive potential of FDG-PET among chemotherapy-treated patients with advanced breast cancer[45, 46].

A study assessing the early metabolic response to neoadjuvant letrozole as defined by FDG-PET examination was conducted among 11 patients with hormone receptor positive early-stage breast cancer. This study demonstrated the correlation between FDG-PET metabolic response at 4 weeks and change in tumor size as measured by ultrasound, pathological response, and change in Ki-67 labelling index as compared to lack of such an FDG-PET metabolic response[47]. Two additional studies provided further evidence supporting the notion that metabolic response assessed by FDG-PET in patients with metastatic breast cancer is predictive of the PFS[48, 49]; one among 22 patients with hormone receptor positive metastatic disease treated with endocrine therapy and the second among 25 patients with early-stage disease treated with neoadjuvant letrozole and bevacizumab. These studies showed that FDG-PET metabolic response was correlated with clinical and/or pathological outcomes.

An exploratory sub-study of the NeoALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization) study, a neoadjuvant trial evaluating combinations of trastuzumab and/or lapatinib with paclitaxel among women with HER2-positive breast cancer, reviewed the role of FDG-PET as a possible biomarker in HER2-positive disease in 77 of the 455 patients included in NeoALTTO[50]. Metabolic changes in the primary tumor in patients with invasive operable breast cancer treated with HER2 blockade were detected as early as after 2 weeks of treatment. Additionally, pathological complete response (pCR) rates were twice as high in PET responders compared to non-responders according to EORTC criteria (41% versus 21% at week 2 and 43% versus 19% at week 6). While this substudy is too small to draw definitive conclusions, FDG-PET early response may be a promising tool particularly for clinical development of new drugs or combinations[51]. Additional evidence for a potential role of FDG-PET as a predictive biomarker has been generated for targeted agents as well, mostly assessing this role in the setting of mTOR inhibition in different tumor types[52-55].

5. Trial objectives and endpoints

5.1. Primary objective

This is a single arm phase II trial for postmenopausal patients receiving fulvestrant and palbociclib for estrogen receptor positive (ER+) / human epidermal growth factor receptor 2 negative (HER2-) metastatic or locally advanced breast cancer who have progressed under previous endocrine therapy. The primary objective is to interrogate in a prospective manner a series of potential biomarkers, which will be assessed for their association with PFS, specifically:

a. gene mutations in a panel of genes assessed on ctDNA, as determined by the AURORA platform

b. gene mutations and gene copy number aberrations of an extended panel of cancer-related genes measured on primary tumors and/or tissue from metastatic biopsies, as determined by the AURORA platform.

c. baseline levels of activity of TK1 as well as early changes in TK1 levels measured during and after one cycle of therapy.

d. gene signatures as inferred by RNA sequencing that will be performed through the AURORA program. In particular, in addition to other gene signatures measuring the activational status of several oncogenic signaling pathways, the following gene signatures with potential predictive utility in terms of sensitivity to CDK4/6 blockade will be assessed, namely:

   i. a gene signature called 'RBsig' consisting of 87 genes that is indicative of loss-of-function of the Retinoblastoma (Rb) pathway[41].

   ii. A gene signature developed at Université libre de Bruxelles consisting of 11 genes that is indicative of the phosphorylation status of CDK4.

5.2. Primary endpoint

Progression-free survival (PFS) is defined as time from treatment initiation until documented disease progression according to RECIST 1.1 criteria (Section 13.12) or death, whichever occurs first.

5.3. Secondary objectives

To evaluate:

- Safety and tolerability, as documented according to NCI CTCAE v4.0
- Disease control, based on RECIST 1.1 criteria

Secondary endpoints are defined in section 18.2.

6. Trial design, duration and termination

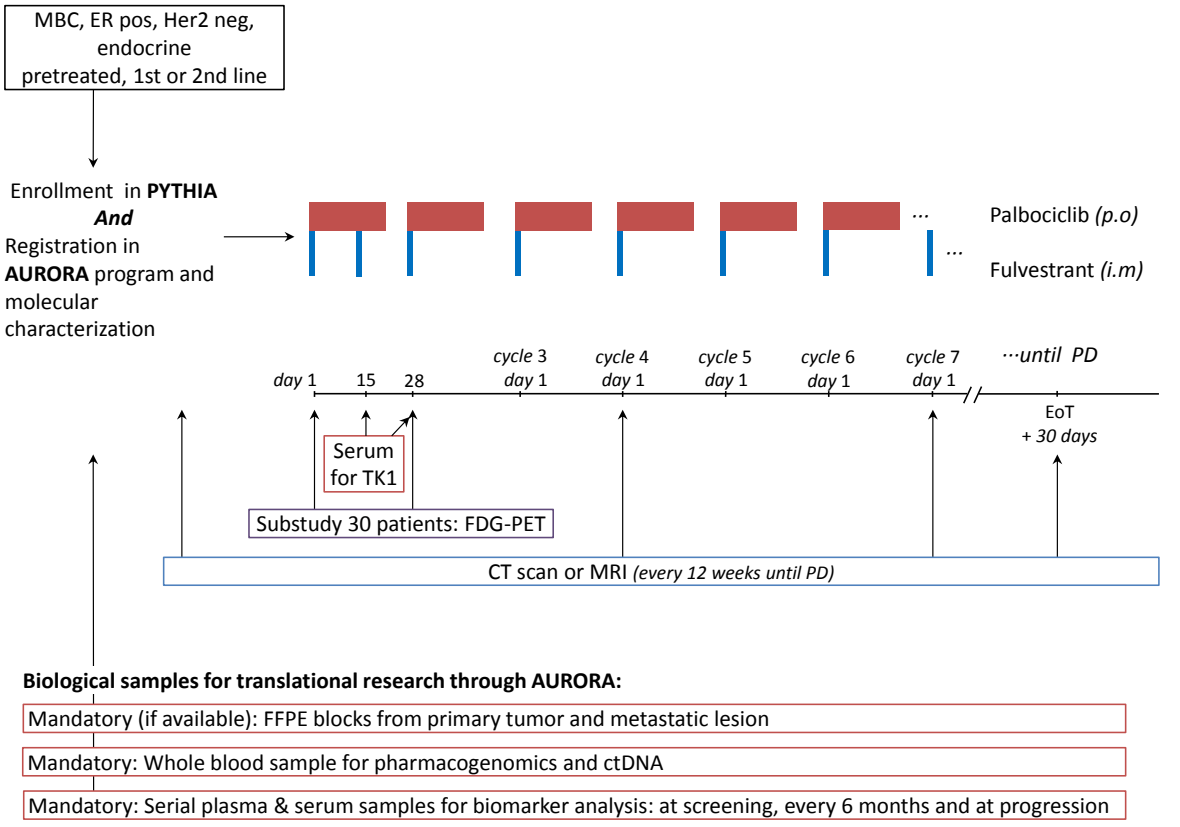
6.1. Trial design

This is an international, multi-center, phase II trial that will enroll postmenopausal women with ER-positive, HER2-negative metastatic or locally relapsed breast cancer progressing under previous endocrine treatment to receive the combination of palbociclib plus fulvestrant.. One line of prior chemotherapy treatment for advanced disease is allowed.

An FDG-PET substudy will be conducted at selected sites on the potential association between metabolic response as assessed through FDG-PET and PFS for 30 patients enrolled in the present study. This FDG-PET evaluation will take place at baseline (max. 2 weeks prior to start of trial treatment), as well as at the end of week 4 (day 25-28).

Patients have to be registered in the AURORA program, which means that tumor samples of both primary and metastatic disease (if available) are being secured and will be centralized in the AURORA bio-repository (Integrated Biobank of Luxembourg (IBBL), with registered office at 6, rue Nicolas Ernest Barblé, L-1210, Luxembourg).

6.2. Trial schema



6.3. Treatment

Fulvestrant 500mg (2 injections of 250mg) will be administered intramuscularly on days 1 and 15 of cycle 1, and then on day 1 (+/- 3 days) of each subsequent 28 days cycle. Palbociclib will be administered orally at a dose of 125 mg per day continuously for 3 weeks followed by

1 week off; repeated at each subsequent cycle of 28 days. Patients will receive the treatment until progression, lack of tolerability, or patient declines further protocol treatment.

6.4. Clinical evaluations

Tumor response will be evaluated according to RECIST version 1.1 (see section 13) using computed tomography (CT) or magnetic resonance imaging (MRI) of the thorax, abdomen and pelvis. For each patient, the same technique must be used to evaluate each lesion through the trial.

Tumor assessments will be performed at screening (prior to start of protocol therapy), and every 12 weeks from start of treatment until progressive disease is observed. Bone scans should be performed as clinically indicated. All patients who are discontinued from the protocol therapy for any reason other than disease progression will continue to have tumor assessments as per the schedule and until the patient has documented disease progression, for up to a maximum of 12 months. Any other radiological assessment should be performed as clinically indicated according to the medical judgment of the Investigator.

6.5. Biological evaluations

Patients that have been consented for the AURORA program for metastatic breast cancer, conducted by BIG, will be candidates for enrollment in the current trial. FFPE material from primary breast tumor and one metastatic lesion will be subjected to next generation sequencing for an extended panel of cancer-related genes through the AURORA program, making available extensive molecular background information.

Blood samples for biomarker analyses will be taken through the AURORA program. In addition, a serum sample will be taken at two weeks and at the end of cycle 1 in view of the thymidine kinase-1 (TK1) assay DiviTum™ .

6.6. Sample size and trial duration

The trial will enroll a total of 120 patients.

Patients will be enrolled by approximately 22 AURORA sites in Belgium, Italy and the United Kingdom.

The enrollment is expected to be completed within 12 months after activation of Amendment 1. Individual patients' trial participation ends with the End of Treatment visit, or a maximum of 12 months after EoT in the absence of tumor progression (Section 10.6). Clinical visits are expected to span approximately 36 months after enrollment of the first patient. The final trial analysis is expected 30-36 months after the inclusion of the first patient under Amendment 1. The end of the trial is defined as last patient last visit and is expected to be 36 months after enrollment of the first patient under amendment 1.

7. Patient selection

7.1. Inclusion criteria

7.1.1. Histologically confirmed breast adenocarcinoma that is metastatic or locally relapsed

disease not amenable to curative therapy.

7.1.2. Female gender

7.1.3. Age ≥ 18 years

7.1.4. Postmenopausal, defined as women with:

- Prior bilateral surgical oophorectomy; or
- Amenorrhea and age ≥ 60 years; or
- Age < 60 years and amenorrhea for 12 or more consecutive months in the absence of alternative pathological or physiological cause (including chemotherapy, tamoxifen, toremifene, or ovarian suppression) and FSH and serum estradiol levels within the laboratory's reference ranges for postmenopausal women.

7.1.5. Endocrine resistant disease, defined as one of:

- Relapse while on adjuvant endocrine therapy;
- Relapse within 12 months after completion of adjuvant endocrine therapy;
- Progression of disease under first line endocrine therapy for metastatic and/or loco-regionally advanced breast cancer.

Note: Patient may have received one prior chemotherapy for advanced or metastatic breast cancer.

7.1.6. ER positive tumor and HER2-negative tumor, as assessed locally.

7.1.7. Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1 (see Table 1 below).

7.1.8. Measurable or non-measurable but evaluable disease according to RECIST 1.1. Bone only disease is allowed. Previously irradiated lesions are deemed measurable only if progression is documented at the site after completion of radiation.

7.1.9. Written Informed Consent (IC) for screening procedures and trial participation must be signed and dated by the patient and the Investigator prior to screening.

7.1.10. Written informed consent to participate in the AURORA program of BIG.

7.1.11. The patient has been informed of and agrees to data transfer and handling, in accordance with national data protection guidelines.

7.1.12. Life expectancy >3 months.

7.1.13. Hematologic status:

- Absolute neutrophil count ≥ 1.5×10^9/L (without growth factor support),
- Platelet count ≥ 100×10^9/L (no transfusion allowed within 2 weeks prior to assessment),
- Hemoglobin ≥ 9 g/dL (transfusion permitted)

7.1.14. Hepatic status:

- Serum total bilirubin ≤ 1.5 × upper limit of normal (ULN). In the case of known Gilbert's syndrome, a higher serum total bilirubin (< 3 × ULN) is allowed.
- AST and ALT ≤ 2.5 × ULN; if the patient has liver metastases, ALT and AST must be ≤ 5 × ULN.

7.1.15. Glucose in normal range, or well-controlled diabetes defined as an HbA1c level ≤ 7.5%.

7.1.16. Renal status:

- Creatinine ≤ 1.5 ×ULN or creatinine clearance > 60 ml/min.

7.1.17. International Normalized Ratio (INR) or Prothrombin Time (PT) ≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulant.

7.1.18. Ability to swallow oral medication.

7.2. Exclusion criteria

7.2.1. Prior use of fulvestrant or any CDK inhibitor.

7.2.2. More than one prior line of chemotherapy for metastatic or locally relapsed disease.

7.2.3. Previous or current non-breast malignancies within the last 5 years, with the exception of in situ carcinoma of the cervix, and adequately treated basal cell or squamous cell carcinoma of the skin.

7.2.4. Known active uncontrolled or symptomatic CNS metastases, carcinomatous meningitis or leptomeningeal disease as indicated by clinical symptoms, cerebral edema, 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,) and are clinically stable off anticonvulsants and steroids for at least 4 weeks before enrollment.

7.2.5. Any of the following in the previous 6 months: myocardial infarction, severe/unstable angina pectoris, ongoing cardiac dysrhythmias of NCI CTCAE grade ≥2, atrial fibrillation of any grade, coronary/peripheral artery bypass graft, symptomatic congestive heart failure (NYHA functional classification ≥III, see Table 2 below), cerebrovascular accident including transient ischemic attack, or symptomatic pulmonary embolism.

7.2.6. QTc exceeding 480msec, family or personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP).

7.2.7. Uncontrolled electrolyte disorders that can reinforce the QT-prolonging effect of the drug (e.g., hypocalcemia, hypokalemia, hypomagnesemia).

7.2.8. Known history of HIV seropositivity. HIV screening is not required at baseline.

7.2.9. Uncontrolled diabetes defined as HbA1c level > 7.5%.

7.2.10. Concurrent disease or familial, sociological or geographical condition that would make the patient inappropriate for trial participation or any serious medical disorder that would interfere with the patient's safety.

7.2.11. Dementia, altered mental status, or any psychiatric condition that would prevent the understanding or rendering of Informed Consent.

7.2.12. Known abnormalities in coagulation such as bleeding diathesis, or treatment with anticoagulants precluding intramuscular injections of fulvestrant; see section 10.5.1 for details.

7.2.13. Treatment with an investigational agent in the 4 weeks before enrollment.

7.2.14. Concurrent treatment with any of the drugs not permitted, i.e. strong CYP3A inhibitors/inducers and drugs known to cause QT interval prolongation; see section 10.5.1 for details.

7.2.15. Adverse events (except alopecia) from previous systemic cancer therapy, radiotherapy or surgery have not recovered to CTCAE v4.0 grade 1 or resolved prior to enrollment.

Table 1. ECOG Performance Status

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

Table 2. NYHA functional classification

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
I	Patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

8. Enrollment

This trial will use a web-based enrollment system. Specific details for enrollment are in the "IBCSG Registration/Randomization Procedures Manual" which is available on the IBCSG website (www.ibcsg.org).

8.1. Patient registration

8.1.1. Verify that the patient has given written informed consent to the AURORA program.

8.1.2. Patient needs to give informed consent to trial participation and screening procedures for the PYTHIA trial.

8.1.3. Verify eligibility (see section 7). Screening procedures need to be done within 28 days before enrollment (maximum within 42 days for imaging).

8.1.4. Access the IBCSG Registration/Randomization System (Registration: Step 1) and provide the requested information as indicated on the Confirmation of Registration (53-A1) Form. The date the Informed Consent Form was signed by the patient and the date signed by the Investigator are both required to complete registration.

The IBCSG Registration/Randomization System will provide the Patient ID (Registration Number) via e-mail.

8.1.5. Submit the Confirmation of Registration (53-A) electronic case report form (eCRF) via iDataFax.

8.2. Registration Help Desk

The IBCSG Data Management Center (located at Frontier Science and Technology Research Foundation (FSTRF)) is responsible for developing and maintaining the IBCSG Registration/Randomization System. The Help Desk includes technical personnel and administrators of the registration programs at the Data Management Center in Amherst, NY, USA.

The Help Desk is available around the clock 7 days per week, except for New Year's Eve, Memorial Day, Independence Day, Thanksgiving Day, Christmas Day.

FSTRF Randomization Help Desk
Frontier Science & Technology Research Foundation (FSTRF)
4033 Maple Rd, Amherst, NY 14226 USA
Phone: +1 716 834 0900 Extension 7301
Fax: +1 716 832-8437
Email: bc.helpdesk@fstrf.org

8.3. AURORA Registration Confirmation

Participation in the PYTHIA trial requires co-registration in the AURORA Trial.

8.3.1. Register the patient to the AURORA program and obtain the AURORA Patient ID Number (for details on the AURORA registration procedures, please refer to the AURORA protocol).

8.3.2. Complete the AURORA Registration Confirmation Form (Form 53-AUR). The AURORA Patient ID Number, date of registration, and AURORA Center Code are required to complete the form.

8.3.3. Submit this form via iDataFax within two weeks of enrollment to PYTHIA.

9. Trial drugs formulation and handling

Palbociclib is the Investigational Medicinal Product (IMP) used in this trial; IMP will be supplied.

Following the marketing authorization for palbociclib in November 2016, and once available in the respective countries, commercial product labelled for clinical trial use may be supplied.

Fulvestrant will also be supplied.

Complete details of the trial drug logistics, distribution, packaging, labeling, storage and handling as well as accountability are described in a separate ***Drug Supply Manual***. This document is available for reference by the pharmacist and trial personnel.

9.1. Palbociclib

9.1.1 Name and Chemical Information:

Palbociclib (also known as PD-0332991)

9.1.2 Chemical Structure:

6-Acetyl-8-cyclopentyl-5-methyl-2-{[5-(1-piperazinyl)-2-pyridinyl]amino}pyrido[2,3-d]pyrimidin-7(8H)-one

9.1.3 Mechanism of Action:

Selective inhibitor of the cyclin-dependent kinases CDK4 and CDK6

9.1.4 Formulation

Refer to the current version of the PD-0332991 Investigator's Brochure and the SPC (Summary of Product Characteristics) for pharmaceutical formulation information.

Palbociclib will be supplied as capsules containing 75 mg, 100 mg, or 125 mg equivalents of palbociclib free base. Vendor will supply the oral drug formulation to sites in High Density Polyethylene (HDPE) bottles containing 75 mg, 100 mg, or 125 mg capsules. The capsules can be differentiated by their size and color (see below).

Table 3. Palbociclib capsule characteristics

Dosage	Capsule color	Capsule length
75 mg	Sunset Yellow/Sunset Yellow	17.3 mm length
100 mg	Caramel/Sunset Yellow	19 mm length
125 mg	Caramel/Caramel	21.2 mm length

9.1.5 Packaging and labeling

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.1.6 Storage and handling

Clinical supplies must be stored at 20°C to 25°C in a secure, limited-access location under the storage conditions specified on the label. The Investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

9.2 Fulvestrant

9.2.1 Presentation

Fulvestrant is used in accordance with the currently approved SPC and regarded as non-investigational medicinal product (NIMPs), in accordance with the applicable EU legislation. Commercial fulvestrant (Faslodex®) will be supplied.

Each vial contains 250 mg.

9.2.2 Storage and handling

Store fulvestrant in refrigerated conditions (2°C – 8°C). All drugs will be stored and handled as per the current version of the product's SPC and the standard hospital procedures. Pharmacy will maintain temperature logs of all storage conditions and comply with hospital pharmacy standard operating procedures.

10 Treatment

10.1 Trial treatments

Trial treatment should start within one week after enrollment. Trial treatments will be administered in 4-week (28-day) cycles until progression, lack of tolerability, or until further protocol treatment is declined (see section 10.6). Fulvestrant can be continued in case of palbociclib cessation due to toxicity. If fulvestrant has been ceased for whatever reasons, palbociclib has to be stopped as well.

Treatment administration should comply with the protocol; compliance will be monitored by the Monitoring Team or Data Management Center. Complete details of dispensation and dosing are recorded on the eCRF.

10.2 Treatment Administration

10.2.1 Fulvestrant administration

Fulvestrant will be administered

- 500 mg intramuscularly on days 1 and 15 of cycle 1, and then
- 500 mg intramuscularly on day 1 of each subsequent 28 day cycle (+/- 3 days).

Fulvestrant 500 mg will be administered intramuscularly into the buttocks slowly (1-2 minutes per injection) as two 250 mg / 5 mL injections, one in each buttock.

Planning the time of fulvestrant administration (e.g., time of the week for first administration; time of the day for each administration) should take trial visit procedures into consideration.

10.2.2 Palbociclib administration

Palbociclib will be administered orally at a dose of 125 mg per day continuously dosed for 3 weeks followed by 1 week off; repeated at each subsequent cycle. The duration of a cycle of treatment is 28 days. A deviation of 1-3 days from this schedule is permitted. On treatment day 1, patients will be provided with trial treatment for self-administration at home. Sufficient capsules should be provided to cover administration until next scheduled visit. The patients will be instructed to take the trial treatment exactly as prescribed, promoting compliance. All dosages prescribed and dispensed to the patient and all dose changes, if applicable, during the trial must be recorded on the Treatment Form (53-PT).

Patients should be instructed to take the palbociclib with food. Concomitant intake of proton pump inhibitors does not significantly affect the overall exposure to palbociclib taken 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 trial drug in the Patient Diary, and to bring the diary to the clinic at each visit. The Investigator should keep a copy in the patient file.

10.3 Dose modifications and delays for fulvestrant

Refer to standard of care guidance or current clinical practice or currently approved fulvestrant European SPC for patients receiving fulvestrant treatment.

No dose adjustment for fulvestrant is permitted. A fulvestrant dose can be skipped a maximum of two times during the whole trial duration for an individual patient. In case a fulvestrant dose cannot be delivered for a third time or two times consecutively then the patient should definitely stop all trial treatment. Treatment delay for fulvestrant-related toxicities will be performed as per the Investigator's best medical judgment, but by no more than 7 days. In the event of a toxicity requiring dosing delay of palbociclib, fulvestrant can also be delayed.

Fulvestrant should not be administered if the platelet count is <50'000/mm^3, and should be delayed until satisfactory recovery of the platelet count, preferably by not more than 7 days.

Every effort should be made to synchronize day 1 of palbociclib and fulvestrant administration.

Patients discontinuing fulvestrant treatment are not allowed to receive palbociclib according to trial protocol.

10.4 Dose delays and modifications for palbociclib

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.

Every effort should be made to synchronize day 1 of palbociclib and fulvestrant administration.

Table 4. Palbociclib dose reduction/delay for hematological toxicities

Note: Table is adapted from SPC table 2, and applies to all hematological adverse reactions except lymphopenia (unless associated with clinical events, e.g. opportunistic infections)

ANC = absolute neutrophil count

CTCAE 4.0 Grade	Dose modification / delay
Grade 1 or 2	No dose adjustment is required
Grade 3	Day 1 of cycle: Withhold palbociclib, repeat complete blood count within 1 week. When recovered to Grade ≤ 2, start next cycle at the same dose Day 14 of first 2 cycles: Continue palbociclib at current dose to complete cycle. Repeat complete blood count on day 21. Consider dose reduction in case of prolonged (> 1 week) recovery from Grade 3 neutropenia or recurrent Grade 3 neutropenia in subsequent cycles.
Grade 3 ANC (500 – <1000 / mm3) + fever ≥ 38°C and/or infection	Withhold palbociclib until recovery to Grade ≤ 2. Resume at next lower dose.
Grade 4	Withhold palbociclib until recovery to Grade ≤ 2. Resume at next lower dose.

Table 5. Palbociclib dose reduction/delay for non-hematological toxicities

(table adapted from SPC table 3)

CTCAE 4.0 Grade	Dose modification / delay
Grade 1 or 2	No dose adjustment is required
Grade ≥ 3	Withhold until symptoms resolve to 1. Grade ≤ 1 2. Grade ≤ 2 (if not considered a safety risk for the patient) Resume at next lower dose

Patients requiring more than two dose reductions will be allowed to receive 75mg/day for 2 weeks after 2 weeks off trial treatment (if, per the Investigator's judgment, such schedule is manageable and preferred; this must be discussed with IBCSG beforehand by contacting ibcsg53_pythia@fstrf.org). Taking palbociclib according to recommendation (i.e., with food) should be reinforced and confirmed. Alternatively, the patient will be discontinued from the trial and entered into the follow-up phase of the trial. All dose modifications/adjustments must be clearly documented in the patient's source notes and the Protocol Therapy Form 53-PT.

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.

Patients discontinuing palbociclib treatment due to treatment-related toxicity may continue on the active treatment phase of the trial receiving fulvestrant monotherapy at the Investigator's discretion.

Table 6. Available dose levels

Dose Level	**Palbociclib for 3 out of 4 weeks**	**Fulvestrant on day 1 of every 28 days cycle**
Starting dose	125 mg/d	2x 250 mg/injection
-1	100 mg/d	2x 250 mg/injection
-2	75 mg/d*	2x 250 mg/injection
-3 (for selected cases only)	Discontinue palbociclib treatment or consider schedule of 75mg/d 2 weeks on / 2 weeks off. This must be discussed with IBCSG beforehand by contacting ibcsg53_pythia@fstrf.org. If 2 weeks on/2 weeks off is not tolerated, then discontinue palbociclib.	

* Palbociclib dose de-escalation below 75 mg/d is not allowed but if considered appropriate the schedule may move to 75mg/day two weeks on followed by two weeks off.

10.5 Concomitant therapy

The following rules should be followed:

- **Anti-cancer** (cytotoxic or endocrine therapy, other than fulvestrant provided within the trial protocol) **or investigational therapy** is not permitted while patients are on trial therapy.
- **Administration of radiotherapy** concurrently with trial medications is not allowed, except for palliative radiotherapy as specified below:

  For patients with bone involvement, it is suggested to institute palliative radiotherapy before trial initiation if possible and clinically appropriate. However, palliative radiotherapy is allowed exclusively for the treatment of painful bone lesions provided that the lesions were known to be present at baseline and the Investigator clearly documents that the need for palliative radiotherapy is not indicative of disease progression.

  In view of the current lack of data about the interaction of palbociclib with radiotherapy, palbociclib treatment should be interrupted during palliative radiotherapy, stopping 1 day before and resuming treatment 1 week after.
- **Concomitant surgery**:

  Caution is advised on theoretical grounds for any surgical procedures during the trial. The appropriate interval of time between surgery and palbociclib required to minimize the risk of impaired wound healing and bleeding has not been determined. Based on the available pharmacokinetic data, stopping palbociclib is recommended at least 7 days prior to elective surgery. Postoperatively, the decision to reinitiate palbociclib treatment should be based on a clinical assessment of satisfactory wound healing and recovery from surgery.
- **Hormone replacement therapy**, topical estrogens (including any intra-vaginal preparations), megestrol acetate and selective estrogen-receptor modulators (e.g., raloxifene) are prohibited.
- The following guidelines are to be followed with respect to administration of **bisphosphonates and/or denosumab** in patients enrolled in the trial:
  - Chronic concomitant bisphosphonate/denosumab therapy for the prevention of bone metastases is not permitted during the trial.
  - Bisphosphonate/denosumab therapy for the treatment of osteoporosis of any grade is permitted during the trial.
  - Bisphosphonate/denosumab therapy at the time of enrollment for the management of bone metastases is recommended as standard of care.
- Other supportive or palliative measures are permitted while patients are on trial therapy, as per physician's choice, including hematopoietic growth factors.

The use of other concomitant medication/therapy judged by the Investigator to be necessary for the care of the patient is permitted. The Investigator should instruct the patient to notify the trial site about any new medications she takes after the start of the trial drug.

The exclusion criteria in section 7.2 describe other medications which are prohibited in this trial. There are no prohibited therapies during the post-treatment follow-up phase.

10.5.1 Prohibited medications:

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

- Strong CYP3A inhibitors/inducers: Palbociclib is metabolized to multiple metabolites in a qualitatively similar manner in rat, dog and human liver microsomes. *In vitro*, palbociclib is primarily metabolized by CYP3A4 enzymes. Co-administration with drugs that are CYP3A inhibitors and inducers may change the plasma concentrations of palbociclib in humans.

  The concurrent use of CYP3A inhibitors, including amprenavir, atazanavir, boceprevir, clarithromycin, conivaptan, delavirdine, diltiazem, erythromycin, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, voriconazole, and grapefruit, grapefruit juice or any product containing grapefruit, are not allowed in the trial.

  The concurrent use of CYP3A inducers, including carbamazepine, felbamate, nevirapine, phenobarbital, phenytoin, primidone, rifabutin, rifampicin, rifampin, rifapentin, and St. John's wort, are not allowed in the trial.

- Drugs known to cause QT interval prolongation are prohibited during the active treatment phase (refer to **Error! Reference source not found.**).

- Anticoagulants: if patient is receiving anticoagulants, refer to the Faslodex Product information[56].

10.5.2 Medications not recommended

The following treatments are not recommended throughout the duration of the active treatment phase. Alternative therapies should be considered whenever possible.

- Moderate CYP3A Inducers: The concurrent use of moderate CYP3A inducers such as bosentan, efavirenz, etravirine, modafinil, and nafcillin, as well as dexamethasone, is not recommended.

- CYP3A Substrates: Palbociclib and its oxidative metabolite, PF-05089326, demonstrated little or no inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6 enzyme activities and thus, showed low potential for CYP-mediated pharmacokinetic drug interactions. However, palbociclib and PF-05089326 caused time-dependent inhibition of CYP3A midazolam 1'-hydroxylase and testosterone 6β-hydroxylase activities with K_i and k_{inact} values for PD-0332991 of 10 µM, 0.036 min-1 and 19 µM, 0.087 min-1 and for PF-05089326 of 7.0 µM, 0.094 min-1 and 6.4 µM, 0.15 min-1, respectively. Therefore,

palbociclib and its metabolite may have the potential for pharmacokinetic drug interactions with compounds for which CYP3A-mediated metabolism constitutes the primary mechanism of clearance. While the clinical significance of this inhibitory effect is yet to be investigated, caution must be exercised in patients receiving palbociclib in combination with drugs that are predominantly metabolized by CYP3A. In particular, co-administration palbociclib with CYP3A4 substrates with narrow therapeutic index including, but not limited to alfentanil, aripiprazole, cyclosporine, ergotamine, fentanyl, halofantrine, pimozide, quinidine, sirolimus, tacrolimus, triazolam, astemizole, cisapride, and terfenadine are not recommended during the active treatment phase of the trial. Alternative therapies should be used when available.

- Chronic immunosuppressive therapies should be avoided, including systemic corticosteroids. Steroids given for physiological replacement, as anti-emetics or inhaled as well as short course of oral/topical steroids given for allergic reactions or asthma flares are allowed.
- The use of herbal medicine is not recommended during the active treatment phase.

10.6 Stop of trial treatment

Duration of therapy will depend on individual response, evidence of disease progression and tolerance.

The treatment of the individual patient will be discontinued in case of:

- Disease progression according to RECIST 1.1. as defined in section 13.
- Unacceptable adverse event(s).
- Intercurrent illness that prevents further administration of trial treatment.
- Patient demonstrates an inability or unwillingness to comply with the treatment regimen and/or trial requirements.
- General or specific changes in the patient's condition which render her unacceptable for further trial treatment in the opinion of the treating Investigator.
- Patient withdraws consent to continue trial treatment.

Note that in the unlikely case the patient does not fulfill AURORA screening requirements (due to failure of more than one sequencing of primary tumor tissue, metastatic tumor tissue and ctDNA), the patient continues trial treatment.

Patients who discontinue treatment for any reason other than objective disease progression will continue to be followed every 12 weeks until documented disease progression, or in the absence of progression, for a maximum of 12 months, as per Section 14.6, and have the end of treatment visit as per Section 14.5.

After the discontinuation of protocol therapy, future therapeutic decisions are at the discretion of the Investigator, with no restrictions. The End of Treatment (EoT) is defined as the last date that the patient has taken trial treatment. In the absence of tumor progression, the patient will continue to be followed for safety and efficacy after this time until documented disease

progression or a maximum of 12 months after stop of treatment. An end of treatment visit will be conducted within 30 days after EoT to report on any adverse events during this period (safety data).

10.7 Removal from the trial

After a patient has been enrolled, she becomes part of the clinical trial population and cannot be removed from the trial for any reason other than a decision by the patient to decline any further participation with the trial requirements and/or to decline further collection of data (see Section 19.5). If the patient discontinues treatment for any of the reasons listed in the prior subsection other than disease progression, she should continue to be followed according to the protocol (see Section 10.6) and eCRFs should be completed as described in Section 17.1.

Patients who have been enrolled but never received any trial treatment for whatever reason (refusal, medical condition etc.) will have to be documented with an end of treatment visit (see section 14.5) and may be replaced.

11 Safety

11.1 Adverse reactions to fulvestrant

Adverse reactions by system organ class and frequency		
Infections and infestations	Common	Urinary tract infections
Immune system disorders	Common	Hypersensitivity reactions
Metabolism and nutrition disorders	Common	Anorexia[a]
Nervous system disorders	Common	Headache
Vascular disorders	Common	Venous thromboembolism[a], hot flushes
Gastrointestinal disorders	Very common	Nausea
	Common	Vomiting, diarrhea
Hepatobiliary disorders	Very common	Increased hepatic enzymes (ALT, AST, ALP)[a]
	Common	Elevated bilirubin[a]
	Uncommon	Hepatic failure[c], hepatitis[c], elevated GGT
Skin and subcutaneous tissue disorders	Common	Rash
Musculoskeletal and connective tissue disorders	Common	Back pain[a]
Reproductive system and breast disorders	Uncommon	Vaginal moniliasis, leukorrhea, vaginal hemorrhage
General disorders and administration site conditions	Very common	Asthenia[a], injection site reactions[b]
	Uncommon	Injection site hemorrhage, injection site hematoma

[a] Includes adverse drug reactions for which the exact contribution of Faslodex cannot be assessed due to the underlying disease.

[b] The term injection site reactions does not include the terms injection site hemorrhage and injection site hematoma.

[c] The event was not observed in major clinical studies (CONFIRM, FINDER 1, FINDER 2, NEWEST). The frequency has been calculated using the upper limit of the 95% confidence interval for the point estimate. This is calculated as 3/563 (where 563 is the number of patients in the major clinical studies), which equates to a frequency category of 'uncommon'.

11.2 Adverse reactions to palbociclib

Table 7, taken from the SPC table 4, reports the adverse reactions from the pooled dataset of 3 randomized studies. The median duration of palbociclib treatment across the pooled dataset was 12.7 months. The adverse reactions are listed by system organ class and frequency category. Frequency categories are defined as: very common (≥1/10), common (≥1/100 to <1/10), and uncommon (≥1/1,000 to <1/100).

The effect of palbociclib on the QT interval corrected for heart rate (QTc) interval was evaluated using time matched electrocardiogram (ECG) change from baseline and pharmacokinetic data in 77 patients with breast cancer. At the recommended dose, no palbociclib relevant effects on QT have been observed.

Table 7. Adverse reactions based on pooled dataset from 3 randomized studies (N=872)

System Organ Class Frequency Preferred Term	All grades %	Grade 3 N (%)	Grade 4 N (%)
Infections and infestations			
Very common			
Infections b	54.7	39 (4.5)	6 (0.7)
Blood and lymphatic system disorders			
Very common			
Neutropenia c	80.6	55.3	10.1
Leukopenia d	45.2	26.1	0.6
Anemia e	27.6	4.4	0.2
Thrombocytopenia f	19.0	1.6	0.3
Common			
Febrile neutropenia	1.6	1.1	0.1
Metabolism and nutrition disorders			
Very common			
Decreased appetite	15.8	0.8	0.0
Nervous system disorders			
Common			
Dysgeusia	8.5	0.0	0.0
Eye disorders			
Common			
Vision blurred	4.4	0.1	0.0
Lacrimation increased	5.7	0.0	0.0
Dry eye	3.6	0.0	0.0
Respiratory, thoracic and mediastinal disorders			
Common			
Epistaxis	8.4	0.0	0.0
Gastrointestinal disorders			
Very common			
Stomatitis g	28.9	0.7	0.0
Nausea	34.2	0.3	0.0
Diarrhea	24.5	1.0	0.0
Vomiting	17.1	0.5	0.0

System Organ Class Frequency Preferred Term	All grades %	Grade 3 N (%)	Grade 4 N (%)
Skin and subcutaneous tissue disorders			
Very common			
Rash h	16.5	0.7	0.0
Alopecia	25.9	Not applic.	Not applic.
Common			
Dry skin	9.4	0.0	0.0
General disorders and administration site conditions			
Very common			
Fatigue	39.2	2.3	0.2
Common			
Asthenia	12.8	1.4	0.0
Pyrexia	12.4	0.1	0.0
Investigations			
Common			
ALT increased	8.0	1.7	0.1
AST increased	8.6	2.5	0.0

a Preferred Terms (PTs) are listed according to MedDRA 17.1.

b Infections includes all PTs that are part of the System Organ Class Infections and infestations.

c Neutropenia includes the following PTs: Neutropenia, Neutrophil count decreased.

d Leukopenia includes the following PTs: Leukopenia, White blood cell count decreased.

e Anemia includes the following PTs: Anemia, Hemoglobin decreased, Hematocrit decreased.

f Thrombocytopenia includes the following PTs: Thrombocytopenia, Platelet count decreased.

g Stomatitis includes the following PTs: Aphthous stomatitis, Cheilitis, Glossitis, Glossodynia, Mouth ulceration, Mucosal inflammation, Oral pain, Oropharyngeal discomfort, Oropharyngeal pain, Stomatitis.

h Rash includes the following PTs: Rash, Rash maculo-papular, Rash pruritic, Rash erythematous, Rash papular, Dermatitis, Dermatitis acneiform, Toxic skin eruption.

11.3 Potential drug induced liver injury

Abnormal values in aspartate transaminase (AST) and/or alanine transaminase (ALT) concurrent with abnormal elevations in total bilirubin that meet the criteria outlined below in the absence of other causes of liver injury are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and should always be considered important medical events.

The threshold for laboratory abnormalities in the case of potential drug-induced liver injury depends on the patient's individual baseline values and underlying conditions. Patients who present with the following laboratory abnormalities should be evaluated further to definitively determine the etiology of the abnormal laboratory values:

- Patients with AST or ALT and total bilirubin **baseline values within the normal range**

- who subsequently present with AST or ALT ≥3 times the upper limit of normal (× ULN)

concurrent with

- a total bilirubin ≥2 × ULN with no evidence of hemolysis and an alkaline phosphatase ≤2 × ULN or not available.

- Patients with pre-existing AST or ALT **baseline values above the normal range**:
  - AST or ALT ≥2 × the baseline values and ≥3 × ULN, or ≥8 × ULN (whichever is smaller)

concurrent with

- total bilirubin ≥2 × ULN and increased by 1 × ULN over baseline or ≥3 × ULN (whichever is smaller) with no evidence of hemolysis and an alkaline phosphatase ≤2 × ULN or not available

The patient should return to the investigational site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history and physical assessment. In addition to repeating AST and ALT, laboratory tests should include albumin, creatine kinase, total bilirubin, direct and indirect bilirubin, gamma-glutamyl transferase, prothrombin time (PT)/INR, and alkaline phosphatase. A detailed history, including relevant information, such as review of ethanol, acetaminophen, recreational drug and supplement consumption, family history, occupational exposure, sexual history, travel history, history of contact with a jaundiced subject, surgery, blood transfusion, history of liver or allergic disease, and work exposure, should be collected. Further testing for acute hepatitis A, B, or C infection and liver imaging (e.g., biliary tract) may be warranted. All cases confirmed on repeat testing as meeting the laboratory criteria defined above, with no other cause for liver function test abnormalities identified at the time should be considered potential Hy's Law cases irrespective of availability of all the results of the investigations performed to determine etiology of the abnormal liver function tests. Such potential Hy's Law cases must be reported as SAEs.

11.4 Drug-drug interactions

Drug interaction studies have not been conducted in humans. Palbociclib is metabolized *in vitro* primarily via CYP3A4. See sections 10.5.1 and 10.5.2 for prohibited and not recommended medications.

The potential for a clinically significant drug-drug interaction between palbociclib and fulvestrant is expected to be very low. There are no known drug-drug interactions.

Palbociclib is metabolized to multiple metabolites in a qualitatively similar manner in rat, dog and human liver microsomes. In vitro, palbociclib is primarily metabolized by Cytochrome P-450 (CYP) 3A4 enzymes.

The routes of elimination for fulvestrant include combinations of different biotransformation pathways including oxidation, aromatic hydroxylation, conjugate with glucuronic acid and/or sulphate. Fulvestrant does not significantly inhibit any of the major CYP isoenzymes, including CYP 1A2, 2C9, 2C19, 2D6, and 3A4 *in vitro*. Studies of co-administration of fulvestrant with midazolam indicate that therapeutic doses of fulvestrant have no inhibitory effects on CYP 3A4 or alter blood levels of drug metabolized by that enzyme. According to

Fulvestrant product information, studies of interaction with rifampicin (CYP3A4 inducer) or ketokonazole (a potent inhibitor of CYP3A4) did not show any clinically relevant modification of fulvestrant PK. Therefore, dosage adjustment is not necessary in patients co-prescribed CYP 3A4 inhibitors or inducers.

Both fulvestrant and palbociclib display a favorable toxicity profile. Therefore, the likelihood that the combination of fulvestrant and palbociclib could cause clinically significant unexpected toxicities is low.

The PALOMA 3 trial has yielded detailed information on adverse events under the combination of fulvestrant and palbociclib, and has shown a good tolerability of the combination. Toxicity will be carefully monitored during trial.

12 Adverse event and serious adverse event reporting

12.1 Adverse event reporting

The main criterion for tolerability is the occurrence of toxicities and adverse events. The severity and causality will be classified according to the NCI CTCAE Version 4.0. The CTCAE is available for downloading on the internet at http://evs.nci.nih.gov/ftp1/CTCAE/About.html. An interactive version can be found at http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx.

An adverse event is defined as any untoward medical occurrence that occurs from the first dose of trial medication until 28 days after all treatment discontinuation, regardless of whether it is considered related to a medication.

Any grade of any observed adverse event should be reported on the Adverse Event Log (53-AE) in iDataFax. Symptoms of the targeted cancer (if applicable) should not be reported as adverse events.

12.1.1 Severity / intensity

The adverse event severity grade provides a qualitative assessment of the extent or intensity of an adverse event, as determined by the Investigator or as reported by the patient. The severity grade does not reflect the clinical seriousness of the event, only the degree or extent of the affliction or occurrence (e.g., severe nausea, mild seizure), and does not reflect the relationship to trial drug.

Severity grade for other adverse events not covered in the toxicity grading scale:

Grade 1 = Mild – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required

Grade 2 = Moderate – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required

Grade 3 = Severe – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible

Grade 4 = Life threatening – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

Grade 5 = Death – the event results in death

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is *not* the same as "serious" which is based on patient/event *outcome* or *action* criteria associated with events that pose a threat to a patient's life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

Note:

- Report the highest grade observed until resolution of the adverse event. In case the grade increases over time, update the grade only (Start date should remain the same).
- Baseline symptoms will be recorded on the Form 53-14 BAE and continuing events and worsening of grade during treatment have to be reported on the 53-14 AE Log.
- Any abnormal laboratory values grade ≥ 3 will have to be documented on the 53-14 AE Log.
- AEs should not be reported in a narrative description, but rather by using the applicable CTCAE v4.0 term.

12.1.2 Causality

The Investigator must determine the relationship between the administration of trial drug(s) and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below:

Not suspected:	The temporal relationship of the adverse event to trial drug(s) administration makes a causal relationship unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.
Suspected:	The temporal relationship of the adverse event to trial drug(s) administration makes a causal relationship possible, and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

12.1.3 Duration

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event.

12.1.4 Action taken

The Investigator will report the action taken with trial drug(s) as a result of an AE or SAE, as applicable (e.g., discontinuation of trial drug(s)) and in case of an SAE report if concomitant and/or additional treatments were given for the event.

12.2 Targeted adverse events

The presence of the following AEs must be indicated on the Adverse Event Log (Form 53-AE):

- Neutrophil count decreased
- Febrile neutropenia
- Anemia
- Platelet count decreased
- Nausea
- Diarrhea
- Pulmonary embolism
- Infections
- Skin and subcutaneous tissue disorders

12.3 Adverse events of special interest (AESIs)

Potential cases of drug-induced liver injury (see section 11.3) are considered AESIs and need to be notified to IBCSG immediately (i.e., within 24h), following the SAE reporting instructions described in Section 12.6, even if not fulfilling a seriousness criterion.

12.4 Otherwise reportable events

Certain types of events, as identified below, are reportable to IBCSG under the reporting processes and requirements for SAEs, even if there is no associated adverse event. These are considered "otherwise reportable events" and generally reflect circumstances that could lead to an increased risk of an adverse event. Like an SAE, an otherwise reportable event is to be reported to IBCSG within 24 hours of awareness and followed up to determine outcome, including the later occurrence of an associated SAE.

12.4.1 Pregnancy and lactation

Pregnancy will be reported within 24h of awareness on the Pregnancy Form (53-PREG-Initial) and on the Serious Adverse Event Form (53-SAE-A) in all cases.

Exposure during lactation will be reported within 24h on the 53–SAE-A Form.

In the event of pregnancy or lactation, all trial treatment must be discontinued.

Follow-up of the pregnancy is mandatory until the outcome has been determined. Outcome will be reported on the Pregnancy Form (53-PREG-Outcome).

12.4.2 Relevant overdose of IMP

An overdose (accidental or intentional) with the IMP is an event suspected by the Investigator or spontaneously notified by the patient and defined as the intake of

- more than 23 capsules during a cycle (e.g. two capsules on more than two days of a cycle, or more than two capsules during the week of break), or
- more than 2 capsules on the same day.

The overdose has to be reported within 24h on the 53–SAE–A Form.

12.5 Serious adverse event (SAE)

12.5.1 Definition

An SAE is defined in general as any undesirable medical occurrence/adverse drug experience that occurs from signature of Informed Consent until 28 days after stopping all trial treatment that, at any dose, results in any of the following:

- fatal (any cause)
- life-threatening
- requires or prolongs inpatient hospitalization
- persistent or significant disability/incapacity
- secondary (non-breast) malignancy
- congenital anomaly or birth defect (including neonatal deaths)
- constitutes an important medical event

Important medical events are defined as those occurrences that may not be immediately life-threatening or result in death, hospitalization, or disability, but may jeopardize the patient or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

After completion of trial treatments, report all SAEs beyond 28 days that are considered at least possibly related to previous trial treatment. Cases of second (non-breast) malignancies and congenital abnormalities are to be regarded as SAEs, regardless of whether they occur during or after trial treatment. These events should be reported during the whole trial duration on the Serious Adverse Event eCRFs (53–SAE–A and 53–SAE–B).

A suspected unexpected serious adverse reaction (SUSAR) is an adverse event that is serious, related to the investigational drug and not listed as a known toxicity of the investigational drug in the Investigator's brochure. All suspected unexpected serious adverse reactions judged by either the Investigator or IBCSG as the sponsor will be reported in accordance with applicable local regulations.

12.5.2 Exceptions to the definition

Hospitalizations occurring under the following circumstances are not considered to be serious adverse events:

- elective surgery
- occur on an outpatient basis and do not result in admission (hospitalization <24h)
- are part of the normal treatment or monitoring of the studied treatment
- progression of disease (by convention, clinical events related to the primary cancer being studied or to the primary cancer progression are not to be reported as SAEs, even if they meet any of the seriousness criteria from the standard SAE definition,

unless the event is more severe than expected and therefore the Investigator considers that their clinical significance deserves reporting).

12.5.3 Causality assessment

The Investigator needs to assess the relationship between protocol treatment and the occurrence of each SAE following the definitions in this table:

Relationship to the protocol treatment	**Description**
suspected	The possibility that the protocol treatment caused the event is deemed definite or probable or possible
not suspected	The possibility that the protocol treatment caused the event is deemed unlikely or unrelated

The Investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, medical history, concurrent conditions, concomitant therapy, other risk factors, and the temporal relationship of the event to the protocol treatment will be considered and investigated.

The decision will be recorded on the SAE Form and if necessary the reason for the decision will also be recorded.

12.5.4 Expectedness assessment / reference safety information

The expectedness assessment is the responsibility of the sponsor of the trial. The expectedness assessment will be performed against the following reference documents:

- For fulvestrant: Summary of Product Characteristics (SPC)
- For palbociclib: latest version of Investigator's Brochure

12.6 Reporting SAEs

Any SAE and any AESI or other reportable event (sections 12.3 and 12.4) occurring in a patient after providing Informed Consent must be reported, including death due to any cause other than progression of breast cancer, which occurs within 28 days following cessation of treatment or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the investigational product. Information about all such events will be collected and recorded on the IBCSG Serious Adverse Event eCRFs (53–SAE–A and 53–SAE–B).

To ensure patient safety, the IBCSG must be informed of each SAE using the procedures described below:

- The Investigator/MD responsible for the patient must complete a Serious Adverse Event (SAE-A) eCRF in English within 24 hours of awareness via iDataFax. A copy is automatically forwarded to the IBCSG Safety Office for medical review.
- Queries may be issued by the IBCSG Safety Office; a timely response by the Investigator to all SAE-related queries is crucial.
- Follow-up information should be completed, via iDataFax, on the Serious Adverse Event (SAE-B) eCRF as early as possible but within 15 days of the initial report, even

if the event reported in the SAE-A eCRF is not yet resolved. If the event is not resolved within 15 days, revise the original Serious Adverse Event (SAE-B) eCRF in iDataFax to report the final resolution.

- All SAEs that have not resolved upon discontinuation of the patient's participation in the trial must be followed until recovered, recovered with sequelae, not recovered (death due to another cause) or death (due to the SAE).
- If a non-serious adverse event becomes serious, this and other relevant follow-up information must also be provided within 24 hours.
- Photocopies of all examinations carried out with the dates on which they were performed should be sent by fax or DFSend into the DataFax system. Care should be taken to ensure that the patient's identity is protected and the Patient ID number is properly included on ALL pages of any reports. For laboratory results, include the laboratory normal ranges. Please also note on each page that the information is "SAE related" so it can be properly categorized in iDF.
- In the event the eCRF system is not working, the SAE Forms can be found in the trial site file or downloaded from the IBCSG trial webpage and sent via fax or DFSend into the DataFax system.

If an SAE (SAE-A and SAE-B Forms) was submitted by fax or DFSend, the original forms and the fax confirmation sheet(s) must be kept at the Participating Center.

The IBCSG will inform Pfizer Pharmacovigilance, AstraZeneca Pharmacovigilance and other appropriate persons about all SAEs within 24 hours of receipt at the IBCSG.

The IBCSG will record the SAE and prepare a monthly SAE report. Principal Investigators will receive the summary report on a monthly basis, and these reports can be found on the IBCSG web site (www.ibcsg.org).

12.7 Occupational exposure

If any patient-care or other personnel comes into contact with the content of palbociclib capsules then this needs to be reported to IBCSG via email to ibcsg53_pythia@fstrf.org.

13 Disease assessment, response and progression (RECIST 1.1)

13.1 Introduction

All enrolled patients will be assessed for disease response and progression according to the revised Response Evaluation Criteria in Solid Tumors (RECIST version 1.1)[57]. In this trial, patients may have measurable or non-measurable disease (see definitions below). Patients will be re-evaluated every 12 weeks until documented progression.

Progression-free survival (primary endpoint) will be assessed using RECIST 1.1 criteria.

13.2 Methods of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during treatment and follow-up. Imaging-based

evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

CT scan is the best currently available and reproducible method to measure lesions selected for response assessment. CT scan should generally be performed using a ≤ 5 mm contiguous reconstruction algorithm. MRI is acceptable for certain situations (e.g., body scans).

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules) and ≥10 mm. In the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT scan is preferable.

Ultrasound is not useful in assessment of lesion size and is not accepted as a method of assessment.

FDG-PET is not foreseen for regular response assessments. It may, however, be used to detect or confirm the appearance of new lesions. Attenuation correction CT scans performed as part of a PET/CT scan frequently show lower resolution; therefore, dedicated CT scans are preferred. However, if the site can demonstrate that the CT scan performed as part of a PET/CT is of the same diagnostic quality as a diagnostic CT scan (with i.v. and oral contrast), then the CT scan portion of the PET/CT can be used for RECIST measurements.

13.3 Measurability of tumor at baseline

13.3.1 Measurable disease

Measurable disease is defined as the presence of at least one measurable lesion.

Measurable lesions:

- **Tumor lesions** must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
  - 10 mm by CT scan (CT scan slice thickness no greater than 5mm).
  - 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
  - 20 mm by chest X-ray.

  Reminder: A lesion in a previously irradiated area is not eligible for measurable disease.
- **Malignant lymph nodes**: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan, assuming the slice thickness is ≤ 5 mm. At baseline and in follow-up, only the short axis will be measured.

13.3.2 Non-measurable disease

Non-measurable disease is defined as lesions or sites of disease that cannot be measured.

Non-measurable lesions/sites of disease and special considerations:

- Small non-nodal lesions (longest diameter < 10 mm in CT scan).
- Small lymph nodes (short axis ≥ 10 and < 15 mm). Lymph nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed as measurable or non-measurable disease.
- Bone lesions. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.
- Leptomeningeal disease
- Ascites
- Pleural or pericardial effusion
- Inflammatory breast disease
- Lymphangitic involvement of skin or lung
- Cystic lesions. Cystic lesions thought to represent cystic metastases may be considered as measurable lesions. However, if non-cystic lesions are present, these are preferred as target lesions.
- Tumor lesions situated in a previously irradiated area, or subjected to other locoregional therapy. Such lesions may be considered measurable if there has been demonstrated progression in the lesion.
- Abdominal masses/abdominal organomegaly identified by physical exam that are not measurable by reproducible imaging techniques.

13.4 Selection of target lesions

Target lesions should be identified, measured and recorded at baseline (Form 53-TEV-B). At baseline, there can be up to a maximum of 5 lesions representative of all involved organs, and up to 2 per organ. Target lesions should be selected on the basis of their size and their suitability for accurate repetitive measurements. A sum of diameters for all target lesions will be calculated and reported as the baseline sum of diameters. Lymph nodes selected as target lesions should always have the short axis recorded. All other lesions should always have their longest diameters recorded. The sum of diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

13.5 Selection of non-target lesions

Non-target lesions should be identified. All other lesions (or sites of disease) not identified as target lesions should also be recorded as non-target lesions at baseline.

For non-target lesions, measurements are not required, but the presence or absence of each should be noted throughout follow-up (Form 53-TEV). It is possible to record multiple non-target lesions as a single item on the eCRF (e.g., "multiple liver metastases").

13.6 Evaluation of target lesions (measurable disease)

All target lesions will be measured at each tumor assessment, and the sum of their diameters will be compared to previous assessments in order to assign the response status as specified below.

- Complete Response (CR): Disappearance of all target lesions. Lymph nodes selected as target lesions must each have reduction in the short axis to <10 mm in order for the response to be considered complete. In this case, the sum of diameters may be >0.
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions taking as reference the baseline sum of diameters.
- Progression (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum recorded on trial. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions (see section 13.8) denotes disease progression.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest sum of diameters recorded on trial.

Note: All target lesions, including lymph nodes, should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). If the radiologist does not feel comfortable assigning an exact measure and reports a lesion as "too small to measure", a default value of 5 mm should be recorded. If a target lesion is thought likely to have disappeared, use "0 mm."

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD.

13.7 Evaluation of non-target lesions

All non-target lesions will be assessed at each tumor assessment, and compared to previous assessments in order to assign the response status as specified below.

- Complete Response (CR): Disappearance of all non-target lesions; lymph nodes selected as non-target lesions must be non-pathological in size (< 10 mm).
- Non-CR/non-PD: Persistence of one or more non-target lesions (non-CR).
- Progression (PD): unequivocal progression of existing non-target lesions. Unequivocal means: comparable in magnitude to the increase that would be required to declare PD for measurable disease, or an overall substantial increase in tumor burden that merits treatment discontinuation.

When no imaging is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesions are evaluated at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD.

13.8 Determination of new lesions

The appearance of any new malignant lesions denotes disease progression. The finding of a new lesion should be unequivocal (i.e., not attributable to differences in scanning technique or findings thought to represent something other than tumor). If a new lesion is equivocal, (e.g., because of its small size) the patient will stay on treatment (if the decision on PD is based on this lesion only). If the repeat scan documents that there is definitely a new lesion, then progression should be declared using the date of the previous scan when the lesion was discovered.

Lesions or sites of disease found in a new location not included in the baseline scan (e.g., brain metastases) are considered new lesions.

Note: The "re-appearance" of a previously "disappeared" target or non-target lesion does not in itself necessarily qualify as PD; this is the case only if the overall evaluation meets the PD criteria, or if the patient was previously in CR.

13.9 Additional considerations

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

13.10 Determination of time point response

Based on the responses of target lesions, non-target lesions, and the presence or absence of new lesions, the overall response will be determined at each tumor evaluation time point, according to Table 8 or Table 9 below.

13.10.1 For patients with measurable disease

Table 8. Measurable Disease - Overall Response

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR / non-PD*	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD

Target lesions	Non-target lesions	New lesions	Overall response
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD
*Non-CR/non-PD should be used rather than SD for categorizing non-target lesions.			

13.10.2For patients with non-measurable disease only

Table 9. Non-Measurable Disease - Overall Response

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD*	No	Non-CR/non-PD*
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
*Non-CR/non-PD should be used rather than SD for categorizing non-target lesions.		

13.11 Determination of best overall response

Best overall response is defined as best response recorded from enrollment across all time points until disease progression. Confirmation of partial or complete response by an additional scan is not requested in this trial. Best overall response will be determined by the IBCSG Head of Medical Affairs, in consultation with the trial chair.

Among patients with measurable disease, the **duration of response** is defined as the time from which the criteria for partial or complete response are first met until disease progression. Among patients with non-measurable disease, the duration of response is defined as the time from which the criteria for CR are first met until disease progression.

13.12 Progression-free Survival

The date of progression is the date that objective progression was first documented. Progression-free survival (PFS) is defined as time from treatment initiation until documented disease progression according to RECIST 1.1 criteria or death, whichever occurs first. For patients without progression, follow-up will be censored at the date of last disease assessment without progression, unless death occurs within a short period of time (12 weeks, corresponding to the interval of tumor re-evaluation) following the date last known progression-free, in which case the death will be counted as a PFS event.

Patients who discontinue treatment prior to documented disease progression (see Section 10.6), including those who initiate non-protocol therapy prior to progression, will be followed for disease progression, for a maximum of 12 months. A new (non-breast) cancer malignancy has to be reported on the 53-14 SAE Forms; such patients must continue to be followed for progression of the original breast cancer.

13.13 Storage of imaging

CT/MRI images must be stored locally in electronic format for potential central review, please follow local guidelines and policies.

14 Clinical and laboratory evaluations and follow-up

14.1 Screening

Patients that have consented to enter the AURORA program (NCT02102165) for metastatic breast cancer, conducted by BIG, will be candidates for enrollment in the current trial. Both primary breast tumor and one metastatic lesion (if available) will be subjected to next generation sequencing for an extended panel of cancer-related genes through the AURORA program, making available extensive molecular background information. The screening regarding the assessment of the eligibility of a candidate to be included in the trial will include securing biomaterial for the AURORA evaluation.

The following examinations should be done within a maximum of 28 days before enrollment. If examinations were done prior to 28 days before enrollment (radiological tumor assessment: max 42 days), they have to be repeated.

14.1.1 Make sure that the patient has consented to AURORA and that her breast cancer has been classified locally as ER-positive and HER2-negative.

14.1.2 Confirmation of post-menopausal status (see 7.1.4).

14.1.3 Obtain informed consent for screening evaluations and trial participation (Informed consent may be obtained earlier than within 28 days before enrollment).

14.1.4 Medical history including details of malignancy: date of diagnosis, primary tumor type characteristics, metastatic tumor type characteristics, prior treatment.

14.1.5 Clinical and radiological (by CT scan or MRI) tumor assessments of Chest/Abdomen/Pelvis should be done preferentially within 28 days prior to enrollment. If a biopsy of the metastatic disease is planned this should be done before treatment start, and the interval from radiological tumor assessment may be extended to max 42 days.

14.1.6 Bone scan and FDG-PET if medically indicated.

14.1.7 Cardiac evaluation: Electrocardiogram (ECG).

14.1.8 Physical examination according to local standards, ECOG Performance Status, height, weight.

14.1.9 Hematology: Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count).

14.1.10 Coagulation function: PT, PTT and INR.

14.1.11 Biochemistry: serum potassium, HbA1c, glucose, sodium, calcium, LDH;

Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;
Kidney function tests: urea, creatinine.

14.1.12 Baseline symptoms and adverse events graded according to CTCAE v4.0 (record on baseline adverse events form 53-BAE). Baseline symptoms and adverse events should be recorded from signature of informed consent to prior to start of treatment.

14.1.13 Record all concomitant medication from 28 days before enrollment to prior to start of treatment.

14.2 Day 1 of every treatment cycle

Investigations marked with * need to be repeated on day 1 prior to start of treatment if not done within 3 days prior to this day.

14.2.1 Physical examination according to local standards, ECOG Performance Status and weight.

14.2.2 * Hematology: Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count).

14.2.3 * Biochemistry: serum potassium, HbA1c, glucose, sodium, calcium, LDH;
Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;
Kidney function tests: urea, creatinine.

14.2.4 Cycle 1 only: Serum sample for TK1 assay 28 days after first dose (between day 26 of cycle 1 and before start of cycle 2 treatment).

14.2.5 Electrocardiogram if medically indicated.

14.2.6 Coagulation function: PT, PTT and INR if medically indicated.

14.2.7 Collection of any adverse event observed in the previous cycle and assignment of appropriate adverse events grade according to the NCI CTCAE Version 4.0.

14.2.8 Record all concomitant medication.

14.2.9 Compliance assessment (check patient diary; hand out new patient diary for next cycle).

14.3 Treatment day 15 (cycle 1)

The Investigator needs to evaluate the status of the patient on day 15 of treatment (=day of second fulvestrant administration in cycle 1). Information to be collected during this visit includes the following:

14.3.1 Physical examination according to local standards.

14.3.2 Assessment of all AEs according to CTCAE v4.0 that have occurred since the day that treatment was initiated.

14.3.3 Coagulation function: PT, PTT and INR if medically indicated.

14.3.4 Complete blood count, if medically indicated.

14.3.5 Compliance assessment (check patient diary).

14.3.6 Record all concomitant medication.

14.3.7 Serum sample for TK1 assay 15 days (+/- 2 days) after first dose

14.4 Tumor assessments

Tumor measurements according to RECIST 1.1 criteria (see section 13) have to be done at baseline, at weeks 12, 24, etc (every 12 weeks ± 2 weeks) until progression:

14.4.1 Clinical and radiological (by CT scan or MRI) tumor assessments.

14.4.2 Bone scan and FDG-PET will be done if clinically indicated at the same time points.

14.5 After discontinuation of trial treatment

End of treatment visit within 30 days after stop of all trial treatment (or at the time of decision to stop the trial treatment if the decision is taken >30 days after last dose):

14.5.1 Physical examination according to local standards, weight and ECOG Performance Status.

14.5.2 Collection of any adverse event and assignment of appropriate adverse events grade according to the NCI CTCAE Version 4.0.

14.5.3 Record all concomitant medication.

14.5.4 Hematology: Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count).

14.5.5 Biochemistry: serum potassium, sodium, HbA1c, glucose, calcium, LDH;
Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;
Kidney function tests: urea, creatinine.

14.5.6 Coagulation function: PT, PTT and INR if medically indicated.

14.5.7 If not done in the 30 days prior to this visit, clinical and radiological tumor assessments by CT scan or MRI, and tumor measurements according to RECIST 1.1 criteria for determination of response (see section 13).

14.5.8 Electrocardiogram

14.6 Follow-up prior to documented disease progression

Every 12 weeks (± 2 weeks) in case trial treatment was stopped prior to documented objective disease progression, until progression is documented, or for a maximum of 12 months after treatment stop. The first visit should take place 12 weeks after the previous tumor evaluation:

14.6.1 Physical examination according to local standards, weight and ECOG Performance Status.

14.6.2 Clinical and radiological (by CT scan or MRI) tumor assessments.

14.6.3 Bone scan and FDG-PET if clinically indicated.

14.6.4 Tumor measurements according to RECIST 1.1 for determination of disease progression.

14.6.5 Serious adverse events up to 28 days after stop of all trial treatment.

14.6.6 Any newly initiated anti-tumor therapy.

15 Biological evaluations

15.1 Introduction and overview

Biological material will be banked as foreseen in the AURORA study (NCT02102165). Two additional blood samples will be taken at two weeks and at the end of cycle 1 for the TK1 assay (see section 15.2.3).

Tumor tissue taken prior to start of treatment in the framework of AURORA will be used to define biomarkers that could identify patients with a high chance of responding to the combination therapy with fulvestrant and palbociclib.

In the AURORA program, a whole blood sample for pharmacogenomics will be taken at inclusion into AURORA; plasma and serum samples will be taken every 6 months and at time of disease progression to monitor the disease and to better understand the contribution of ctDNA to the evolution of the disease.

For successful inclusion in the AURORA program, the successful sequencing of at least 2 out of these 3 biospecimens is required.

15.2 Translational research

The goals of the translational research will be to determine mechanisms and biomarkers for efficacy of and resistance to the combination of fulvestrant and palbociclib.

15.2.1 Planned correlative studies:

Within the AURORA program, next generation sequencing will be performed for a panel of 411 cancer-related genes. Additionally, RNA sequencing data will become available at a later time point. Mutations will be assessed in a panel of genes on ctDNA. Based on this data, the following correlative studies will be performed:

- gene mutations in a panel of genes assessed on ctDNA
- The association of mutations and gene copy number aberrations of an extended panel of cancer related genes assessed in the primary tumor with the clinical outcome of the women enrolled in the present trial.
- The association of mutations and gene copy number aberrations of the same panel of cancer related genes assessed in a metastatic lesion with the clinical outcome of the women enrolled in the present trial.
- The association of mutations in a panel of genes assessed in ctDNA with the clinical outcome.

- The potential predictive value of gene signatures as inferred by RNA sequencing in regards to sensitivity towards the investigational palbociclib/fulvestrant treatment.

15.2.2 Pharmacogenomics:

The 9ml whole blood sample that is collected from each patient within the AURORA program will be analyzed in order to be able to differentiate germline from somatic genetic mutations during the analyses.

15.2.3 Thymidine Kinase 1 assay

Thymidine kinase-1 (TK1) is an enzyme that plays a critical role in the cell cycle and synthesis of DNA. All human cells express the TK1 enzyme during normal cell division through E2F-dependent transcription, but only small amounts of TK1 are released into the serum. Due to uncontrolled cell growth and higher rates of replication compared to normal tissues, solid tumors can secrete pathological levels of TK1 that can be detected in serum with the DiviTum™ technology (Biovica International, Sweden). Given that TK1 expression is E2F-dependent, there is a scientific rationale that TK1 will correlate well with CDK4 inhibitors like palbociclib. The CDK4 inhibitors prevent cellular DNA synthesis by prohibiting progression of the cell cycle from G1 into the S phase. The cell synthesizes TK1 during the S phase of cell division. If the CDK-inhibitor is active in a particular tumor, it is expected that this would be reflected by changes in serum TK1 levels and thereby TK1 can be used as a potential, non-invasive, efficacy biomarker for clinical benefit.

The sampling requirements are described in the AURORA "Laboratory Manual: Procedures for tissue and blood samples collection, storage and shipment" and in the additional sheet entitled "IBCSG 53-14 / BIG 14-4 PYTHIA Serum Sample Instructions". Besides the samples taken for AURORA, two serum samples specifically reserved for the TK1-assay should be taken on day 15 (+/- 2 days) of cycle 1 and between day 26 of cycle 1 and before any cycle 2 treatment, at the latest on day 35.

15.2.4 Not yet specified translational research

The use of the biological material for future research not outlined in this protocol will be under the guardianship of BIG on behalf of the AURORA Steering Committee. As part of the AURORA Informed Consent process, patients are asked to indicate whether they agree to donate their sample for not yet specified future research. The patient's decision is recorded on the AURORA Consent Form.

Translational research proposals not outlined in this protocol will be assessed by the PYTHIA steering committee and the AURORA steering committee for merit and feasibility.

Biomarkers that are published in the future and considered to be of relevance can then also be assessed in the context of this trial.

16 FDG-PET substudy

16.1 Introduction

Palbociclib combined with endocrine treatment has exhibited promising antitumor activity among patients with ER+, HER2- metastatic breast cancer. However, there is no predictive

biomarker associated with either sensitivity or resistance to this promising agent. Selecting patients who will achieve a benefit and thus justify the burden of treatment is of utmost importance.

FDG-PET/CT offers a possible solution to early identification of patients who will not benefit from treatment with fulvestrant and palbociclib. Thus a substudy within the PYTHIA phase II trial evaluating the use of FDG uptake as a biomarker in patients with ER+/HER2- metastatic breast cancer treated with palbociclib plus fulvestrant will investigate the question of whether patients who will NOT derive benefit from this combination of therapy can be selected early in the therapeutic process using FDG-PET/CT metabolic response, thus minimizing unnecessary toxicities in these patients.

16.2 Study objectives

16.2.1 Primary Objective

To evaluate if early metabolic response (MR) using FDG-PET/CT is associated with progression free survival (PFS) in patients with ER+, HER2– metastatic breast cancer treated with fulvestrant plus palbociclib.

16.2.2 Secondary Objective

To evaluate if early metabolic response (MR) using FDG-PET is associated with disease control rate (DCR) in patients with endocrine resistant ER+, HER2– metastatic breast cancer treated with fulvestrant plus palbociclib.

16.3 Study design

A subset of up to 45 patients enrolled by selected sites in Belgium into the PYTHIA trial will enter the FDG-PET/CT substudy to assess early FDG-PET/CT response as a predictor of lack of treatment response to fulvestrant and palbociclib. Because only patients with a metabolically measurable lesion at baseline will continue with the substudy and be statistically analyzed, it is expected that up to 45 patients will need to be enrolled in order to have the targeted number of 30 patients with both a baseline and a day 28 scan. Patients who do not have a metabolically measurable lesion at baseline will not have a day 28 scan.

Screening, treatment and assessment of these patients, including tumor evaluations with CT or MRI as described in Section 14, will be performed as described in the current protocol for the PYTHIA trial population. In addition, for patients participating in the substudy:

- FDG-PET/CT will be done at baseline (within 2 weeks prior to start of trial treatment) and on day (25-)28 (at end of first cycle of treatment, before start of cycle 2 treatment). The 53-PET form must be completed at Baseline and updated at day 28 with information regarding the FDG-PET/CT.
- A blood sample (5mL) will be taken at the same time points for more accurate estimation of the blood glucose level.

In order to ensure image quality and reproducibility, standardization and harmonization, the participating FDG-PET/CT centers are required to have FDG-PET/CT Accreditation through EARL (EANM Research Ltd.) and need to comply with the requirements of continued quality control (QC) of acquisition and reconstruction on a regular basis as outlined in EARL's

accreditation manual. More information can be obtained via the EARL's web site: http://earl.eanm.org.

16.4 Definitions

This study is aimed at early identification of metabolic non-responders. FDG-PET/CT response will use the following methodology based on EORTC and PERCIST criteria.

Response assessment will be performed centrally by 2 independent and blinded PET-CT experts. Consensus reading will be organized in case of discordant assessments. All analyses will be organized and coordinated by a central Imaging Core Lab (Orilab).

Definition of metabolic target lesions on FDG PET/CT:

Target lesions are defined on the baseline FDG-PET/CT based on all of the following criteria:

a. Lesion with a very high probability of malignancy (all lesions potentially corresponding with a benign pathology should not be selected); AND
b. Size ≥1.5 cm (preferably in anatomic images); AND
c. FDG-PET avid lesion with uptake above the background liver uptake as follows:
   Metabolically measurable lesion, i.e. with a marked accumulation of FDG, at least 1.5-fold greater than liver SUV mean + 2 SDs (in 3-cm spherical ROI in normal right lobe of liver). If liver is abnormal, target lesion should have uptake > 2.0 × SUV mean of blood pool in 1-cm-diameter ROI in descending thoracic aorta)

Definition of FDG-PET-CT response:

Both EORTC-based and PERCIST-based response classification methodologies will be performed:

a. ***EORTC-based response assessment***: Dominance Method [58]

   Patient-based classification of metabolic response within 4 classes:

Class 1: All target lesions do respond (reduction of SUV_{max} of more than 25% seen in all lesions).

Class 2: Mixed response with majority of whole body tumor load that responds.

Class 3: Mixed response with majority of whole body tumor load that does not respond.

Class 4: All target lesions do not respond OR presence of a progressive lesion (i.e., increase of SUV_{max} of more than 25% in a known lesion or appearance of a measurable (target) new PET lesion).

b. ***PERCIST-based response assessment [59]***

   A maximum of 10 target lesions (maximum of 2 per organ) will be identified. If more than 2 target lesions within one organ are available: only 2 lesions with the highest SUV will be defined as target lesions.

   SUV_{peak} at baseline and on day 28 of trial treatment will be calculated for all target lesions.

Significant lesion response is defined as a relative decrease of SUV_{peak} of more than 30%, therefore patient will be classified as follows:

- Metabolic responder patient (MRP): the difference between the SUV_{peak} of the hottest lesion at baseline and during treatment is more than 30%;
- Metabolic NON responder patient (MNRP): patient that does not fulfil the definition of MRP.

The primary method for defining response is the EORTC-based dominance method.

16.5 ORILAB – Imaging CoreLAB:

FDG-PET images generated in this study are managed by the Oncology Related Imaging coreLAB (ORILAB) from the Institute Jules Bordet.

16.5.1 Standardization

To assure that centers performing FDG-PET for the PYTHIA PET sub-study follow the same acquisition procedures, ORILAB will provide them a ***Standard Procedures Imaging Manual*** (SPIM). The SPIM describes the procedures involved in patient preparation, data transfer, image analysis and storage.

16.5.2 Data encoding

The PYTHIA FDG-PET substudy imaging data will be encoded in an Acquisition electronic report form (AeCRF) and sent by the FDG-PET substudy centers through the electronic data capture tool (EDC) provided by ORILAB. The tool will also guarantee automatic study images anonymisation.

16.5.3 Quality Control/Quality Assurance - QA/QC

The encoded imaging data will be verified and study images will be visually checked to assure compliance with imaging protocol as described in SPIM and absence of artifacts. If non-compliances or errors are detected queries will be sent to the participating center.

16.5.4 Central Review

Imaging data that successfully passed the QA/QC process will be reported and managed by ORILAB central review. To that, the predefined imaging analysis tools and a review electronic clinical report form (ReCRF) will be provided to the two PET experts to capture the required imaging information. The PYTHIA FDG-PET substudy images will be kept in ORILAB Picture Archiving and Communication System (PACS).

17 Data submission

We will conduct the trial according to the ICH Good Clinical Practice (GCP) guidelines. Keeping accurate and consistent records is essential to a cooperative trial. The following forms are to be submitted at the indicated times by the participating institutions for each patient:

17.1 Case report forms schedule

Forms	***Description/Name***	***Forms Submission*** ***ALL data should be completed in iDataFax (iDF) (unless otherwise specified)***
Informed Consent Form	Consent to participation in clinical trial and biologic material submission	Obtain before registration and keep with patient records as documentation (hard copy only).
Registration and Enrollment		
53-A	Confirmation of Registration Form	Complete in iDF after you have registered the patient in the IBCSG Registration/ Randomization System and to confirm eligibility. Patient will be available in iDF **within 24 hours** of successful registration.
53-AUR	Confirmation of registration into AURORA Form	Complete in iDF after you have enrolled the patient in the IBCSG Registration/ Randomization System.
Baseline		
53-H	History Form	Complete in iDF within 1 week of enrollment.
53-BAE	Baseline Adverse Events and Symptoms Form	Complete in iDF within 1 week of enrollment.
53-CCT	Concomitant Treatment Log	Complete in iDF each time a treatment is started, amended, or ended, including treatments within 28 days prior to start of trial treatment (excluding prior treatment for primary/metastatic breast cancer).
53-TEV-B	Tumor Evaluation Baseline Form	Complete in iDF within 1 week of enrollment.
During trial treatment		
53-PT	Protocol Therapy Form	Complete in iDF at the end of each cycle until treatment stops.
53-AE	Adverse Events Log	Complete/review in iDF at the end of each treatment cycle to report an event has started, grade has worsened, or event has resolved, including events observed up to 28 days after treatment stops.
53-CCT	Concomitant Treatment Log	Review the original CCT Log in iDF at the end of each cycle. Update any current medications if needed and add new medications taken, including medications taken up to 28 days after treatment stops.
53-TEV	Tumor Evaluation Form	Complete in iDF at weeks 12, 24, then every 12 weeks until progression.
Follow Up (after discontinuation of trial treatment, up to documented progression)		
53-EoT	End of Treatment Form	Complete in iDF 30 days after **ALL** protocol treatment stops.
53-AE	Adverse Events Log	Review the original AE Log in iDF 28 days after the last dose of trial treatment. Update any events if needed and add new adverse events .
53-CCT	Concomitant Treatment Log	Review the original CCT Log entries in iDF 28 days after the last dose of trial treatment. Update/add any treatments.
53-TEV	Tumor Evaluation Form	*If* patient discontinued trial treatment prior to documented progression: every 12 weeks until progression, for a maximum of 12 months after all treatment stops.
53-E	Follow-up Form	*If* patient discontinued trial treatment prior to documented progression: every 12 weeks until progression, for a maximum of 12 months after all treatment stops. Visit should be done in conjunction with tumor evaluation.

Forms	*Description/Name*	*Forms Submission* *ALL data should be completed in iDataFax (iDF) (unless otherwise specified)*
Event Driven		
53-SAE-A	Serious Adverse Event/Adverse Event of Special Interest Form A - Initial report	Complete in iDF within 24 hours of the SAE awareness. If iDF is not available, fax the form within 24 hours to DataFax.
53-SAE-B	Serious Adverse Event/Adverse Event of Special Interest Form B - Follow-up report	Complete in iDF within 15 days of the initial report (53-SAE-A). If event is not resolved in 15 days, update 53-SAE-B again at the time of resolution.
53-COC	Change of Consent Form	Complete in iDF if there is any change in patient's consent to participate in the trial, see Section 19.5.2.
53-E-Death	Death Form	Complete in iDF if a patient dies, if patient is still in active follow-up.
53-PREG (Initial)	Pregnancy Form – Initial report	Complete in iDF if a patient becomes pregnant while on trial treatment.
53-PREG (Outcome)	Pregnancy Form – Outcome report	Complete in iDF at the outcome of pregnancy.

The iDF User Manual and the Data Managers' Manual for this trial contain instructions for completing and submitting forms using the iDataFax system.

17.2 Signing and submitting forms

An Authorization Log (see section 17.5) should be completed at each Participating Center to identify the persons who are authorized to complete CRFs.

CRFs should be completed on-line in iDataFax. Reports (lab, pathology, etc.) and any other non-CRF data will need to be sent to the DataFax system via fax or DFSend. Full instructions on submitting forms will be available on the IBCSG website (www.ibcsg.org). Also available on the website is a list of fax numbers that are available for faxing CRFs.

17.3 Data management

Data collected in this trial will be submitted to the IBCSG Data Management Center in Amherst, NY, USA. The Data Management Center will process the data and will generate queries and forms requests. The Data Quality Control Office will oversee overall data submission and query resolution. The IBCSG Coordinating Center in Bern, Switzerland will provide medical review and summary of SAEs. The IBCSG Statistical Center in Boston, MA, USA will perform the data analysis.

17.4 Investigator Site File

Each Participating Center should keep documentation about this trial in an Investigator Site File (ISF). Please arrange the documentation in the order foreseen in the ISF index which will be provided by IBCSG. The following documents should be included (list is not complete):

- Protocol and appendices
- Activation letter
- Accrual reports
- Amendments

- Copy of signed Protocol Signature Pages
- Sample CRFs including blank SAE Forms
- Data Managers' Manual
- Obvious Corrections Document and Signature Page
- Registration/Randomization System Manual
- iDataFax (iDF) Manual
- Drug Supply Manual
- SPIM (Belgian sites only)
- Patient information and Informed Consent templates approved by Ethics Committee
- Palbociclib Investigator's Brochure and updates
- Fulvestrant SPC
- Ethics Committee (and Health Authority, if applicable) approval of protocol, Patient Information Sheet and Informed Consent, amendments
- Ethics Committee review of SAE, Investigators' alert, and other documents
- Correspondence with Ethics Committee and Health Authority (if applicable)
- Certificate of clinical trial insurance
- Agreement with IBCSG
- Center Activation email(s) from IBCSG Data Management Center (protocol and amendments, if any)
- Correspondence with / Information issued by IBCSG Coordinating Center, Data Management Center
- SAE Reports sent from IBCSG Data Management Center
- Normal laboratory values/reference ranges
- Laboratory Certifications
- CV of Principal Investigator and Co-Investigators, GCP certificates
- Trial Training Certificates issued by IBCSG Center Training Office
- Documentation of any training done internally (e.g.,by use of IBCSG Training Confirmation Log)
- Authorization Log
- Center Information Sheet
- Patient identification log (see section 17.6)
- Drug shipment records

- Drug accountability log (including certificates of destruction if applicable)
- Temperature logs
- Weblink to ICH GCP guidelines/Declaration of Helsinki and updates
- Audit certificates / monitoring follow-up letters

17.5 Authorization log

The Principal Investigator (PI) should identify the other members of the Clinical Trial Team who are supervised by the PI and approved to provide information in CRFs, queries, etc. Instructions for completing the Authorization Log can be found in the Authorization Log Manual, posted on the IBCSG website. All changes need to be communicated to IBCSG by updating and e-mailing the authorization log.

17.6 Patient identification log

No patients' names should be used in CRFs or any other documentation transmitted to IBCSG central offices. The only item used to identify a patient is the Patient ID (Registration Number). It is therefore imperative that the local data manager keep an identification log for all patients entered in this trial including:

- Patient's name
- Patient ID issued by the Registration/Randomization System
- Date of birth
- Date of enrollment

18 Statistical considerations

This is a single arm phase II trial for postmenopausal patients receiving fulvestrant and palbociclib for ER+ / HER2- metastatic or locally advanced breast cancer who have progressed under previous endocrine therapy. The primary objective is to interrogate in a prospective manner a series of potential biomarkers, which will be assessed for their association with PFS. A total of 120 patients will be enrolled.

18.1 Primary Objective

A series of different molecular markers will be assessed for their association with the PFS of patients receiving fulvestrant plus palbociclib treatment. The primary efficacy endpoint of PFS is defined in Section 13.12.

18.1.1 Design and Sample Size Determination

Enrollment of 120 patients is expected to proceed over 12 months (approximately 10 patients per month), with an additional 12 months of follow-up after the last patient enrolled. The median PFS on palbociclib plus fulvestrant is expected to be in the range of 9 to 10.5 months, with about 67% of patients having documented PFS events (~80 events) at the time of analysis. For sample size / power considerations we conservatively assessed the simplified

situation wherein PFS is compared between groups of patients with and without a certain biomarker. This could be mutations or gene copy number aberrations in pre-treatment ctDNA or tumor-derived DNA as determined by the AURORA next generation sequencing platform, a gene signature as inferred by RNA sequencing, or a pre-treatment circulating marker level or change in that marker after 1 cycle of trial treatment. The assessment calculated the detectable hazard ratios with 80% power for alternative scenarios in which all 120 patients have biomarker data or conservatively in which ~80% (N=90) patients have biomarker data; and on the basis of nominal two-sided α=0.05 level test and α=0.001 level test as a rough consideration of multiple testing (Table X). For example, we expect approximately 40% of patients' primary tumors to show cyclin D1 amplification and ER mutations in approximately 15-20% of patients' metastatic tumors. The statistical analysis plan will re-estimate power considerations based on the observed accrual pattern, overall treatment hazard ratio and overall average hazard, and with consideration of multiplicity of testing. The sample size of 120 was selected to have 80% power to detect a HR of 2.0 for biomarker with 30-50% prevalence (two-sided α=0.05).

Detectable hazard ratios (HR) with 80% power for the difference in PFS distributions between two biomarker groups (i.e., presence vs. absence), for a range of prevalence of the biomarker, two alpha levels and two analyzable sample sizes.

Analyzable Sample Size	2-sided α-level	Biomarker Prevalence					
		5%	10%	15%	20%	30%	50%
		Detectable HR					
N=120	0.05	4.2	2.85	2.4	2.2	2.0	1.9
	0.001	8.3	4.65	3.65	3.2	2.75	2.5
N=90	0.05	5.25	3.35	2.75	2.5	2.2	2.1
	0.001	>10	5.9	4.5	3.8	3.2	2.9

Calculations based on George SL, Desu MM. Planning the size and duration of a clinical trial studying the time to some critical event. Stats Med 2000;19:441-452[60].

18.1.2 Analysis of Primary Endpoint

On the basis of information that accumulates external to the trial during the years of enrollment and follow-up, the final statistical analysis plan will delineate hypothesis testing-focused versus exploratory analyses, and the approaches to multiple testing and interpretation for each set of analyses. The Statistical Analysis Plan (SAP) will identify a few selected biomarkers as hypothesis testing-focused analysis to replicate or validate the clinical validity of the biomarker for its association with PFS, for future assessment as predictive biomarkers; in such analyses, the cutpoints or functional forms of the biomarker values would be pre-specified.

As a general approach, PFS will be compared between biomarker groups or according to continuous biomarker values using by a stratified log-rank test using stratification factors defined for randomization. The hazard ratio with two-sided 90% CI will be estimated using a stratified Cox proportional hazards modeling, with estimation of hazard ratio with two-sided confidence intervals (CI). The distribution of PFS will be summarized for each treatment group using the method of Kaplan-Meier; median PFS with two-sided 95% confidence interval (CI) will be summarized, as well as other quartiles or t-month PFS times with CIs.

18.2 Secondary Objectives

18.2.1 Safety and tolerability

Adverse events (AE) will be collected using CTCAE v4.0. The maximum grade of each targeted AE while on treatment will be determined, and the frequencies summarized and tabulated according to grade and treatment assignment, with two-sided exact binomial 95% CIs. Table 10 shows the half-width of an asymptotic two-sided 95% CI for a range of possible true AE rates, with a sample size of 90 patients randomized to palbociclib with fulvestrant.

Table 10. Confidence Intervals (CI) for AE Rates (%) in Experimental Group (N=90)

True AE rate (%)	5%	10%	20%	30%	40%	50%
95% CI width	±4.5%	±6.2%	±8.3%	±9.5%	±10.1%	±10.3%

18.2.2 Disease Control

Disease control is defined as best overall response of CR or PR, or SD (or non-CR/non-PD in the case of non-measurable disease only) lasting for at least 24 weeks, measured from enrollment until first documentation of progressive disease. Disease control will be summarized as proportion with two-sided exact binomial 95% CI.

18.2.3 Overall survival

Patients will not be followed for overall survival. However, at time of analysis, survival status of the patients will be available from AURORA and overall survival from time of treatment start will be summarized using the method of Kaplan-Meier.

18.3 Definitions of Trial Populations

Included population: All enrolled patients.

Treated population: All patients who initiate treatment. Safety population: All patients receiving at least one dose of trial treatment will be included in assessments of safety and tolerability.

18.4 Interim Analyses

Interim analyses for efficacy or futility are not planned.

18.5 Accrual

The overall accrual goal of this trial is 120 patients. We anticipate that the accrual rate will be approximately 10 patients per month over 12 months.

18.6 Data and Safety Monitoring

The trial will be presented for review to the IBCSG DSMC at each of their semi-annual meetings. Accrual, safety and accumulation of PFS events will be monitored.

19 Ethical aspects, regulatory approval, and patient informed consent

The Investigator will ensure that this trial is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The trial must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline (January 1997) or with local law if it affords greater protection to the patient. For trials conducted in the EU/EEA countries, the Investigator will ensure compliance with the EU Clinical Trial Directive (2001/20/EC).

19.1 Ethical Review Board/Ethics Committee

All protocols and the patient Informed Consent forms must have the approval of a properly constituted committee or committees responsible for approving clinical trials. The Ethics Review Board (ERB) / Institution Review Board (IRB) written, signed approval letter/form must contain approval of the designated Investigator, the protocol (identifying protocol title and version number), and of the patient Informed Consent. Documentation of Ethics Committee approval(s) must be sent to the IBCSG Data Management Center prior to enrollment of the first patient. The IBCSG Ethics Committee also approves the protocol and reviews it annually.

Any modifications made to the protocol will be reviewed by the IBCSG Ethics Committee and must also be submitted to the appropriate ERB/IRB for information or approval in accordance with local procedures and regulatory requirements and to Health Authorities if required.

Once approved or acknowledged by the appropriate ERB/IRB and by the Health Authorities (if required), the Investigator shall implement the protocol modifications. Protocol modifications for urgent safety matters may be directly implemented following the instructions of IBCSG.

19.2 Regulatory approval procedures

If applicable, in addition to the approval of the Ethics Committee according to national legislation, the protocol, other protocol-related documents including patient information and Informed Consent and other documents as required locally must be submitted to and be approved by the health authority. Documentation of health authority approval must be sent to the IBCSG Data Management Center prior to Participating Center activation.

19.3 Protection of human patients

The IBCSG has an Office for Human Research Protection (OHRP) Federal Wide Assurance (FWA00009439) and follows all of the policies and procedures that are part of that assurance. All potential patients for this trial will receive a full explanation of the trial, its purpose,

treatments, risks, benefits, and all of the other items listed in section 19.4. Additional institution-specific sections should be added to Appendix I as needed.

The medical record must be available for review by the IBCSG monitors and audit team and regulatory authorities as described in section 20.6.

Serious Adverse Event (SAE) Reports are distributed monthly. In addition they are available on the IBCSG website (www.ibcsg.org) for participating Centers.

19.4 Informed Consent

Informed Consent for each patient will be obtained prior to initiating any trial procedures in accordance with the "IBCSG Patient Information Sheet and Informed Consent" (See Appendix I). One signed and dated copy of the Informed Consent must be given to each patient and the original copy must be retained in the Investigator's trial records. The Informed Consent form must be available in the case of data audits. Verification of signed Informed Consent for both the PYTHIA trial and the AURORA program and the date signed are required for enrollment to this trial.

The "Declaration of Helsinki" recommends that consent be obtained from each potential patient in biomedical research trials after the aims, methods, anticipated benefits, and potential hazards of the trial, and discomfort it may entail, are explained to the individual by the physician (http://www.wma.net/en/30publications/10policies/b3/index.html). The potential patient should also be informed of her right to not participate or to withdraw from the trial at any time. The patient should be told that material from her tumor will be stored and potentially used for additional studies not described in this protocol.

If the patient is in a dependent relationship to the physician or gives consent under duress, the Informed Consent should be obtained by an independent physician. By signing this protocol, the Investigator agrees to conduct the trial in accordance with GCP and the "Declaration of Helsinki."

The IBCSG recognizes that each institution has its own local, national, and international guidelines to follow with regard to Informed Consent. Therefore, we provide a template information sheet and Informed Consent form (Appendix I), which can be downloaded and edited to incorporate information specific to your institution (see www.ibcsg.org). The template Patient Information Sheet and Informed Consent (PIS/IC) has been written according to ICH guidelines which state the Informed Consent should adhere to GCP and to the ethical principles that have origin in the "Declaration of Helsinki". The final version should receive the Institutional Review Board/ Local Ethics Committee approval in advance of its use. Centers should send their locally modified PIS/IC to the IBCSG Data Management Center for review and approval before submitting to their Ethics Committee.

19.5 Premature withdrawal

19.5.1 Cessation of trial treatment

Patients have the right to refuse further trial treatment at any time during the trial. Patients may also be withdrawn from trial treatment at any time at the discretion of the Investigator

due to an adverse event, or based on any other relevant medical condition. Such patients will remain in the trial. The patient will continue to be documented according to protocol.

19.5.2 Withdrawal of consent

Patients have the right to withdraw consent for further trial participation at any time without having to specify the reason. The data recorded up to the time point of withdrawal will continue to be evaluated in the trial.

Withdrawal of consent should be documented in both the medical records and in the eCRF (Form 53-COC). For the patient's safety, an end of treatment visit should be performed and documented in the eCRF.

20 Governance and Administrative Considerations

20.1 Insurance

IBCSG will contract the appropriate liability insurance for this trial. Patients who suffer injuries due to the trial should report them immediately to their physician. The local Center should report all alleged claims immediately to the IBCSG.

20.2 Steering Committee

A Steering Committee will be constituted for this trial. The primary responsibilities of the Steering Committee are twofold. First, the Steering Committee is responsible for maintaining the scientific integrity of the trial, for example, by recommending changes to the protocol in light of emerging clinical or scientific data from other trials. Second, the Steering Committee is responsible for the translation of recommendations of the IBCSG Data and Safety Monitoring Committee into decisions. Membership will include BIG and IBCSG officials, trial chair and co-chairs, trial statisticians, representatives from some Participating Centers, and one representative from Pfizer Inc.

General partition of responsibilities:

The Steering Committee has the authority to make and implement any final decisions, such as substudies of the trial or amendments to the trial protocol, and may recommend the termination/early termination of the trial.

The IBCSG Foundation Council and the BIG Executive Board decide on the termination/early termination of the trial.

20.3 Data and Safety Monitoring Committee (DSMC)

The trial will be presented for review to the IBCSG Data and Safety Monitoring Committee (DSMC) at each of their semi-annual meetings. Accrual, safety and accumulation of PFS events will be monitored.

20.4 Publication of trial results

IBCSG and BIG will publish the results of the trial based on the final trial report.

20.5 Premature discontinuation of the trial

The trial may be discontinued early in parts or completely if the information on the trial treatment leads to doubt as to the benefit/risk ratio.

The trial can be terminated at any time if the authorization and approval to conduct the Study is withdrawn by ethics committee or regulatory authority decision, insufficient accrual, emerging new data impacting the scientific value of the trial or ethical grounds.

20.6 Quality Assurance

The IBCSG conducts trials according to the ICH GCP guidelines. The Trial IBCSG Data Manager reviews each CRF. In addition, the IBCSG Medical Reviewer reviews each case at specific timepoints. The IBCSG conducts periodic audit visits to ensure proper trial conduct, verify compliance with GCP, and perform source data verification.

The Investigator should ensure that source documents are made available to appropriately qualified personnel from IBCSG or its designees, or to health authority inspectors after appropriate notification.

At regular intervals during the clinical trial, the Center will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review trial progress, Investigator and patient compliance with clinical trial protocol requirements and any emergent problems. These monitoring visits will include but not be limited to review of the following aspects: patient Informed Consent, patient recruitment and follow-up, SAE documentation and reporting, AEs with pre-specified monitoring documentation and reporting, AE documentation, trial treatment administration, patient compliance with the regimens, drug accountability, concomitant therapy use and quality of data.

20.7 Protocol adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. Under no circumstances should the Investigator contact IBCSG or personnel monitoring the trial to request approval of a protocol deviation, as no deviations are permitted. The Investigator should document and explain any deviations from the approved protocol and promptly report them to IBCSG and to the EC concerned in accordance with the applicable EC policies and procedures. If the Investigator feels a protocol deviation would improve the conduct of the trial this must be considered a protocol amendment, and unless such an amendment is developed and activated by IBCSG and approved by the IRB/IEC/REB it cannot be implemented. All protocol deviations will be documented.

20.8 Data protection

A unique Patient Identification (ID)/Registration Number will be assigned by the IBCSG Registration/ Randomization System to each patient registered into the trial. The names of the patients will not be disclosed to the IBCSG.

Only the Patient ID will be used to identify a patient on the eCRF. Identification of patients must be guaranteed at the Participating Center. In order to avoid identification errors, Centers should keep a Patient Identification Log containing the patients' name, year of birth, and the Patient ID allocated by IBCSG.

Regulatory authorities and the pertinent Ethics Committee (ERB/IRB) may have access to patient data on-site. IBCSG audit or monitoring personnel will also have access to such data on-site.

20.9 Record Retention

The Center must retain all essential documents according to ICH GCP. This includes copies of the patient trial records, which are considered as source data, patient Informed Consent statement, laboratory printouts, drug inventory and destruction logs, and all other information collected during the trial. These documents are to be stored until at least 15 years after the termination of the trial. IBCSG guarantees access and availability of the data entered into iDataFax for at least 15 years after the termination of the trial.

Longer retention may be required for Participating Centers according to national regulations.

In the event that the Principal Investigator retires or changes employment, custody of the records may be transferred to another competent person who will accept responsibility for those records. Written notice of such transfer has to be given to IBCSG and the local Ethics Committee at least one month in advance.

21 Confidentiality

The protocol, CRFs and other protocol-related documents are confidential and are the property of the IBCSG.

22 References

1. Global Cancer Facts & Figures 2008. Atlanta, GA: American Cancer Society,2008.
2. Curtis C, Shah SP, Chin SF et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012; 486: 346-352.
3. Perou CM, Sorlie T, Eisen MB et al. Molecular portraits of human breast tumours. Nature 2000; 406: 747-752.
4. Sorlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001; 98: 10869-10874.
5. Sorlie T, Tibshirani R, Parker J et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003; 100: 8418-8423.
6. Sotiriou C, Neo SY, McShane LM et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A 2003; 100: 10393-10398.
7. Banerji S, Cibulskis K, Rangel-Escareno C et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012; 486: 405-409.
8. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature 2012; 490: 61-70.
9. Ellis MJ, Ding L, Shen D et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature 2012; 486: 353-360.
10. Shah SP, Roth A, Goya R et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012; 486: 395-399.

11. Stephens PJ, Tarpey PS, Davies H et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012; 486: 400-404.
12. Zardavas D, Baselga J, Piccart M. Emerging targeted agents in metastatic breast cancer. Nat Rev Clin Oncol 2013; 10: 191-210.
13. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
14. Bertoli C, Skotheim JM, de Bruin RA. Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol 2013; 14: 518-528.
15. Arnold A, Papanikolaou A. Cyclin D1 in breast cancer pathogenesis. J Clin Oncol 2005; 23: 4215-4224.
16. Keyomarsi K, Tucker SL, Buchholz TA et al. Cyclin E and survival in patients with breast cancer. N Engl J Med 2002; 347: 1566-1575.
17. Desmedt C, Ouriaghli FE, Durbecq V et al. Impact of cyclins E, neutrophil elastase and proteinase 3 expression levels on clinical outcome in primary breast cancer patients. Int J Cancer 2006; 119: 2539-2545.
18. Mittendorf EA, Liu Y, Tucker SL et al. A novel interaction between HER2/neu and cyclin E in breast cancer. Oncogene 2010; 29: 3896-3907.
19. Scaltriti M, Eichhorn PJ, Cortes J et al. Cyclin E amplification/overexpression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. Proc Natl Acad Sci U S A 2011; 108: 3761-3766.
20. Caldon CE, Sergio CM, Kang J et al. Cyclin E2 overexpression is associated with endocrine resistance but not insensitivity to CDK2 inhibition in human breast cancer cells. Mol Cancer Ther 2012; 11: 1488-1499.
21. Finn RS, Dering J, Conklin D et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 2009; 11: R77.
22. Dean JL, Thangavel C, McClendon AK et al. Therapeutic CDK4/6 inhibition in breast cancer: key mechanisms of response and failure. Oncogene 2010; 29: 4018-4032.
23. Knudsen ES, Knudsen KE. Tailoring to RB: tumour suppressor status and therapeutic response. Nat Rev Cancer 2008; 8: 714-724.
24. Dublin EA, Patel NK, Gillett CE et al. Retinoblastoma and p16 proteins in mammary carcinoma: their relationship to cyclin D1 and histopathological parameters. Int J Cancer 1998; 79: 71-75.
25. Ertel A, Dean JL, Rui H et al. RB-pathway disruption in breast cancer: differential association with disease subtypes, disease-specific prognosis and therapeutic response. Cell Cycle 2010; 9: 4153-4163.
26. Michaud K, Solomon DA, Oermann E et al. Pharmacologic inhibition of cyclin-dependent kinases 4 and 6 arrests the growth of glioblastoma multiforme intracranial xenografts. Cancer Res 2010; 70: 3228-3238.
27. Barretina J, Taylor BS, Banerji S et al. Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy. Nat Genet 2010; 42: 715-721.
28. Schwartz GK, LoRusso PM, Dickson MA et al. Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (Schedule 2/1). Br J Cancer 2011; 104: 1862-1868.
29. Flaherty KT, Lorusso PM, Demichele A et al. Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. Clin Cancer Res 2012; 18: 568-576.
30. Leonard JP, LaCasce AS, Smith MR et al. Selective CDK4/6 inhibition with tumor responses by PD0332991 in patients with mantle cell lymphoma. Blood 2012; 119: 4597-4607.

31. Dickson MA, Tap WD, Keohan ML et al. Phase II trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma. J Clin Oncol 2013; 31: 2024-2028.
32. Finn RS, Crown JP, Lang I et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16: 25-35.
33. Finn Rs, Martin M, Rugo HS et al. Palbociclib and Letrozole in Advanced Breast Cancer. NEJM 2016; 375.
34. Turner NC, Ro J, Andre F et al. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. N Engl J Med 2015.
35. Cristofanilli M, Turner NC, Bondarenko I et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016; 17: 425-439.
36. Turner NC, Ro J, Andre F et al. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. N Engl J Med 2015; 373: 209-219.
37. Harbeck N, Iyer S, Turner N et al. Quality of life with palbociclib plus fulvestrant in previously treated hormone receptor-positive, HER2-negative metastatic breast cancer: patient-reported outcomes from the PALOMA-3 trial. Ann Oncol 2016; 27: 1047-1054.
38. Di Leo A, Malorni L. Polyendocrine treatment in estrogen receptor-positive breast cancer: a "FACT" yet to be proven. J Clin Oncol 2012; 30: 1897-1900.
39. Witkiewicz AK, Ertel A, McFalls J et al. RB-pathway disruption is associated with improved response to neoadjuvant chemotherapy in breast cancer. Clin Cancer Res 2012; 18: 5110-5122.
40. Herschkowitz JI, He X, Fan C, Perou CM. The functional loss of the retinoblastoma tumour suppressor is a common event in basal-like and luminal B breast carcinomas. Breast Cancer Res 2008; 10: R75.
41. Malorni L, Piazza S, Ciani Y et al. A gene expression signature of Retinoblastoma loss-of-function is a predictive biomarker of resistance to palbociclib in breast cancer cell lines and is prognostic in patients with ER positive early breast cancer. Oncotarget 2016; 7: 68012-68022.
42. O'Leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol 2016; 13: 417-430.
43. Patnaik A, Rosen LS, Tolaney SM et al. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors. Cancer Discov 2016; 6: 740-753.
44. Zardavas D, Maetens M, Irrthum A et al. The AURORA initiative for metastatic breast cancer. Br J Cancer 2014; 111: 1881-1887.
45. Dose Schwarz J, Bader M, Jenicke L et al. Early prediction of response to chemotherapy in metastatic breast cancer using sequential 18F-FDG PET. J Nucl Med 2005; 46: 1144-1150.
46. Jansson T, Westlin JE, Ahlstrom H et al. Positron emission tomography studies in patients with locally advanced and/or metastatic breast cancer: a method for early therapy evaluation? J Clin Oncol 1995; 13: 1470-1477.
47. Ueda S, Tsuda H, Saeki T et al. Early metabolic response to neoadjuvant letrozole, measured by FDG PET/CT, is correlated with a decrease in the Ki67 labeling index in patients with hormone receptor-positive primary breast cancer: a pilot study. Breast Cancer 2011; 18: 299-308.

48. Forero-Torres A, Saleh MN, Galleshaw JA et al. Pilot trial of preoperative (neoadjuvant) letrozole in combination with bevacizumab in postmenopausal women with newly diagnosed estrogen receptor- or progesterone receptor-positive breast cancer. Clin Breast Cancer 2010; 10: 275-280.
49. Mortazavi-Jehanno N, Giraudet AL, Champion L et al. Assessment of response to endocrine therapy using FDG PET/CT in metastatic breast cancer: a pilot study. Eur J Nucl Med Mol Imaging 2012; 39: 450-460.
50. Baselga J, Bradbury I, Eidtmann H et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012; 379: 633-640.
51. Gebhart G, Gamez C, Holmes E et al. 18F-FDG PET/CT for early prediction of response to neoadjuvant lapatinib, trastuzumab, and their combination in HER2-positive breast cancer: results from Neo-ALTTO. J Nucl Med 2013; 54: 1862-1868.
52. Aide N, Kinross K, Cullinane C et al. 18F-FLT PET as a surrogate marker of drug efficacy during mTOR inhibition by everolimus in a preclinical cisplatin-resistant ovarian tumor model. J Nucl Med 2010; 51: 1559-1564.
53. Brepoels L, Stroobants S, Verhoef G et al. (18)F-FDG and (18)F-FLT uptake early after cyclophosphamide and mTOR inhibition in an experimental lymphoma model. J Nucl Med 2009; 50: 1102-1109.
54. Fuereder T, Wanek T, Pflegerl P et al. Gastric cancer growth control by BEZ235 in vivo does not correlate with PI3K/mTOR target inhibition but with [18F]FLT uptake. Clin Cancer Res 2011; 17: 5322-5332.
55. Wei LH, Su H, Hildebrandt IJ et al. Changes in tumor metabolism as readout for Mammalian target of rapamycin kinase inhibition by rapamycin in glioblastoma. Clin Cancer Res 2008; 14: 3416-3426.
56. Product Information: Faslodex®, fulvestrant (injection). AstraZeneca Pharmaceuticals, Wilmington, Delaware (PI revised 07/2002, reviewed 12/2002). In.
57. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45: 228-247.
58. Hendlisz A, Golfinopoulos V, Garcia C et al. Serial FDG-PET/CT for early outcome prediction in patients with metastatic colorectal cancer undergoing chemotherapy. Ann Oncol 2012; 23: 1687-1693.
59. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. J Nucl Med 2009; 50 Suppl 1: 122S-150S.
60. George SL, Desu MM. Planning the size and duration of a clinical trial studying the time to some critical event. J Chronic Dis 1974; 27: 15-24.