Study Title	**Multicenter, first-line metastatic open-label prospective phase II trial evaluating the combination of Palbociclib (CDK 4/6 Inhibitor) and hormone therapy (Letrozole or Anastrozole) in women with luminal, HER2 negative advanced breast cancer: Evaluation of the prediction of individual treatment efficacy using infrared laser spectroscopy analysis on liquid biopsies (Quantum Optics).**
Project Number	**ICRG 0201**
Version and Date	**Version 9.0, June 2021**
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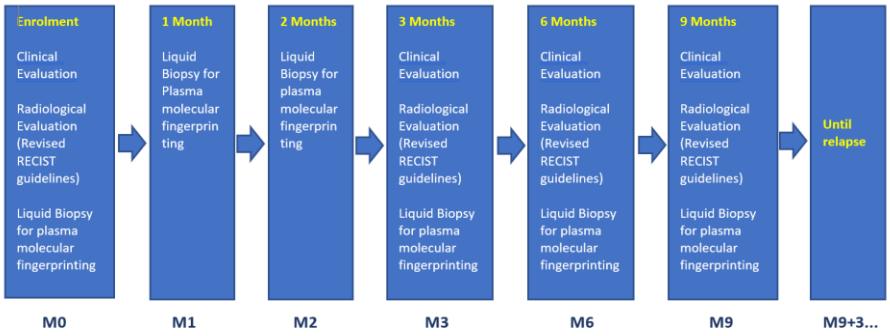
2. SYNOPSIS

Study Title	**Multicenter, first-line metastatic open-label prospective phase II trial evaluating the combination of Palbociclib (CDK 4/6 Inhibitor) and hormone therapy (Letrozole or Anastrozole) in women with luminal, HER2 negative advanced breast cancer: Evaluation of the prediction of individual treatment efficacy using infrared laser spectroscopy analysis on liquid biopsies (Quantum Optics).**
Clinical Phase	Phase II
Study Centers	6-10 cancer centers.
Study Population	Women with luminal, HER2 negative Stage IIIB/IV Breast Cancer.
Study Objective(s)	**Primary Objective** To assess the rate of objective clinical response, according to the revised RECIST guidelines for the combination of Palbociclib + Aromatase Inhibitors (AI). To evaluate the value of infrared laser spectroscopy analysis on liquid biopsies in terms of prediction of the efficacy/resistance of the combination (Palbociclib + Aromatase Inhibitors (AI)) on an individual basis. **Secondary Objectives** ● To evaluate Progression-Free Survival (PFS). ● To assess clinical benefit (CR/PR/Stabilization>24 weeks). ● To assess the 'objective' clinical benefit (CR/PR/Minor response [> 0%] > 24 weeks). ● To evaluate the modification of the individual molecular fingerprinting during the course of the treatment. ● To investigate the role of individualized molecular fingerprinting to predict early progression (>6 months). ● To investigate the role of individualized molecular fingerprinting to predict progressionin age-matched patients with an initial response. ● To evaluate safety and tolerability. ● To perform Benchmark analysis, using Raman Spectroscopy.
Study Design	This is a multicenter, open-label phase II study.

	Eligible pre/peri-menopause* and post-menopausal patients, based on inclusion/exclusion criteria, will be prospectively treated with a combination of Palbociclib + Aromatase Inhibitors (AI). This combination will continue until progression. Treatment response will be evaluated every 3 months using clinical and radiological assessments (Revised RECIST guidelines). Patients will undergo liquid biopsies for plasma molecular fingerprinting by the Quantum Optics technology at baseline (before trial treatment), after each treatment cycle for the first three months and then every 3 months until progression along with clinical and radiological assessments every 3 months. *with oophorectomy for pre/peri- menopausal patients (surgery or Analogs of LHRH). After progression patients will be treated at Principal Investigator (PI)'s discretion.
Visit Schedule and Assessments	**Flow Chart**  See also Appendix 1, 2 & 3 for Assessments, liquid biopsy, and SOPs.
Number of Patients	Eighty (80) patients will be enrolled according to eligibility criteria.
Inclusion Criteria	To be enrolled in the study, patients should meet the following inclusion criteria: 1. Written informed consent before beginning specific protocol procedures including expected cooperation of the patients for the treatment and follow-up must be obtained and documented according to the local regulatory requirements. 2. Postmenopausal women or pre/peri-menopausal with Surgical oophorectomy (preferred) or Analogs of LHRH for pre/peri-menopausal patients). 3. Performance status < 3 (according to WHO criteria). 4. Histologically confirmed breast cancer (Luminal A or B).

	5. Estrogen Receptor positive (ER > 1%). 6. HER-2 negative (score 0 or 1 by immunochemistry), FISH negative if IHC score 2. 7. Clinical stage IIIb & IV. 8. Either: a) Women with De novo advanced luminal HER2 negative advanced breast cancer without other prior systemic treatment for advanced disease. b) Women with luminal HER2 negative advanced breast cancer either with secondary resistance (relapse after 2 years of adjuvant hormone therapy or within 12 months of completion of adjuvant HT) or sensitivity to adjuvant HT (relapse > 12 months after completion of adjuvant HT). 9. Measurable or evaluable disease. 10. Hematology: ● Neutrophil count ≥ 1.5 G/L, ● Platelet count ≥ 100 G/L, ● Leucocyte count > 3.0 G/L, ● Hb> 9g/dl. 11. Hepatic function: ● Total bilirubin ≤ 1.5 times the upper normal limit (UNL), ● ASAT ≤ 2.5xUNL, ● ALAT ≤ 2.5xUNL, ● Alkaline phosphatase ≤ 2.5 times the upper normal limit (UNL). 12. Renal function: ● Serum creatinine ≤1.5xUNL (and if Serum creatinine >1.5xUNL, creatinine clearance ≥40 mL/min). 13. Metabolic function: ● Serum calcium ≥ lower limit of normal. 14. Negative pregnancy test (urine or serum) within 7 days before registration for all women of childbearing potential. Patients of childbearing potential must implement adequate non-hormonal contraceptive measures during study treatment. 15. Patients with negative Human Immunodeficiency Virus (HIV) and/or Hepatitis B and/or Hepatitis C results.
Main Exclusion Criteria	To be enrolled in the study, patients should meet the following exclusion criteria: 1. Male patients. 2. Her-2 positive tumors or unknown HR/HER-2 status. 3. Triple-negative breast cancer (ER<1%). 4. Pregnant or breast-feeding women, or those who plan to become pregnant within 6 months' post-treatment. 5. No willingness to use highly effective methods of contraception (per institutional standard) during treatment and for 6 months post-treatment.

	6. Any form of breast cancer other than those described in the inclusion criteria, particularly inflammatory and/or loco-regional disease (stages I, II & IIIa). 7. Non-evaluable tumor. 8. Bilateral breast cancer. 9. Patients with a history of other cancer, except *in situ* cervical cancer or baso-cellular skin cancer, considered cured. 10. Patient has another disease, which is deemed incompatible with the inclusion in the protocol. 11. Heart, kidney, medullary, respiratory or liver failure. 12. Clinically significant cardiovascular disease (including myocardial infarction, unstable angina, symptomatic congestive heart failure, serious uncontrolled cardiac arrhythmia) at baseline. 13. History of interstitial lung disease e.g. pneumonitis or pulmonary fibrosis or evidence of interstitial lung disease at baseline. 14. Acute urinary infection, ongoing hemorrhagic cystitis at baseline. 15. Uncontrolled diabetes. 16. Symptomatic or progressive disorder of the central nervous system (CNS) at baseline. 17. Patients with positive Human Immunodeficiency Virus (HIV) and/or Hepatitis B and/or Hepatitis C results. 18. Significant psychiatric abnormalities. 19. History of hypersensitivity to studied treatment or excipients. 20. Known previous or ongoing abuse of narcotic drug, other medication or alcohol. 21. Any investigational agent within 30 days before initiation of study treatment. 22. Patient unwilling or unable to comply with study requirements.
Route and Dosage Form	● Palbociclib 125 mg daily per os (3 weeks on and 1 week off) with dose adaptation according to safety profile. ● Aromatase Inhibitors (AI)- Letrozole 2.5mg or Anastrozole 1mg daily per os
Duration of Treatment	Treatment combination period: until progression.
Treatment regimens and pre-medications	Surgical oophorectomy (preferred) or Analogs of LHRH for pre/peri-menopausal women (e.g. Goseriline 3.6 mg subcutaneously every 4 weeks). Duration & dosage of treatment will be adapted in case of toxicity.

	Prevention/ treatment of osteopenia/osteoporosis is recommended as per standard clinical practice.
Primary Endpoints	Rate of objective clinical response, according to the revised RECIST guidelines for the combination of Palbociclib + Aromatase Inhibitors (AI). Sensitivity and specificity of infrared laser spectroscopy analysis on liquid biopsies to predict the efficacy/resistance of the combination (Palbociclib + Aromatase Inhibitors (AI)) on an individual basis.
Secondary Endpoints	● Progression-Free Survival (PFS). ● Clinical benefit (CR/PR/Stabilization>24 weeks). ● 'Objective' clinical benefit (CR/PR/Minor response [> 0%] for > 24 weeks). ● Modification of the individual molecular fingerprinting during the course of the treatment. ● Modification of individualized molecular fingerprinting to predict progression in patients with initial response and objective clinical benefit. ● Modification of individualized molecular fingerprinting to predict progression in age-matched patients with initial response and objective clinical benefit. ● Modification of individualized molecular fingerprinting to predict progression (< 6 months and > 6 months). ● Safety and tolerability. ● Modification of individualized molecular fingerprinting to predict progression obtained from Raman spectroscopy as a benchmark analysis.
Safety Endpoints	● Incidence and frequency of adverse events, serious adverse events. ● Proportion of patients s (%) who prematurely discontinued study treatment. ● Proportion of patients s (%) who prematurely discontinued study treatment due to AEs.
Sample Size Considerations	Considering the response rates (RR) reported in PALOMA 2 trial (overall response rate/ORR: 42% and ORR for measurable disease: 55%, we expect a 50% Objective Response Rate. Considering the initial published data of infrared laser spectroscopy analysis on liquid biopsies comparing plasma molecular fingerprint profiles between a control population without breast cancer and a population with breast cancer with a sensitivity of 98% and a specificity of 97%, we hypothesize that the probability of response to Palbociclib + Aromatase Inhibitors (AI) + will be 50% and the sensitivity to differentiate responders to non-responders by infrared laser spectroscopy analysis on liquid biopsies (prediction of efficacy) will have a sensitivity of 97%. Based on the above hypothesis, the sample size calculated using alpha 5% and power 95%, is N=80 patients to be enrolled and treated.

Statistical Methods and Data Analysis	**Populations:** ● The Intent-to-Treat (ITT) population will consist of all patients enrolled in the study and treated with combination Palbociclib + Aromatase Inhibitors (AI). ● The Full-Analysis (FA) population will consist of all patients enrolled in the study and treated with combination Palbociclib + Aromatase Inhibitors (AI), without major protocol deviations. ● The safety population will consist of all patients who received any study drug and had at least one post-baseline safety assessment. **Statistical Methodology**: Qualitative variables will be described by frequency and percentage. A comparison of the proportion between the two groups of responders (responders and non-responders) will be made using a chi-square test. The 95% confidence intervals of the different response rates will be given. Quantitative variables will be described by the mean, standard deviation, median, maximum and minimum. Time-dependent parameters will be computed by the Kaplan-Meier method to take censored data into account. The Kaplan-Meier product-limit method will be used to estimate the PFS and OS. Cox's proportional hazards regression analysis will be performed for response rates, clinical benefits, PFS. All tests of hypotheses will be one-sided. Confidence intervals of the median survival will be calculated using the method of Simon. Demographic data and baseline characteristics will be presented for the ITT population. The analysis of efficacy endpoints will be performed on the FA population.. The safety analysis will be performed using the safety population. Patients with major protocol deviations will also be described. **The methodology of computational analysis of "biological profiles at n variables".** **Batteries of Algorithmic tests definition:** A "non-hierarchical deep data mining" approach is proposed with: - An analysis with the support vector machines with a polynomial nucleus, which will give, during the analysis of treatment responders/non-responders, highly precise analyses.

	- Age stratifications when applicable. - Presence of sub-stratifications in different age groups. This study will be the first program exploring the adjunction of the Quantum Optics technology on liquid biopsies to define individual 'molecular fingerprinting profiles' to predict the individual therapeutic effects of Palbociclib combined with AI (plus ovarian function suppression (OFS) for pre/peri-menopausal patients) in Luminal, hormone receptor-positive and HER2 negative advanced breast cancer.. Batteries of algorithmic tests will integrate the thousands of variables obtained by Quantum Optics and will correlate the 'individual molecular profiles at n variables' to a binary question (efficacy or not of the combination of Palbocicilb plus AI). This approach is introducing the concept of singularity, breaking from the classic concept 'one size fits all'.

3. List of Abbreviations

AI	Aromatase inhibitors
AE	Adverse Events
ALAT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
ASAT	Aspartate Aminotransferase
BIRD	Broadband Infrared Diagnostics
CDK	Cyclin-Dependent Kinases
CR	Complete Response
CRF	Case Report Forms
CNS	Central Nervous System
CTA	Clinical Trial Agreement
CTCAE	Common Terminology Criteria for Adverse Events
EBC	Early Breast Cancer
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
ER	Estrogen Receptors
FA	Full Analysis
GCP	Good Clinical Practice
Hb	Hemoglobin
HER2	Human Epidermal growth factor Receptor 2
HR	Hormone Receptor
HT	Hormone Therapy
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IHC	ImmunoHistoChemistry
ICRG	International Cancer Research Group
IRB	Institutional Review Board
IR	Infrared
ITT	Intent To Treat
KSA	Kingdom of Saudi Arabia
LHRH	Luteinizing Hormone-Releasing Hormone
LMU	Ludwig-Maximilians-Universität München
MBC	Metastatic Breast Cancer
MedDRA	Medical Dictionary for Regulatory Activities
MPQ	Max-Planck-Institut für Quantenoptik
MRI	Magnetic Resonance Imaging
OFS	Ovarian Function Suppression
ORR	Overall Response Rate
OS	Overall Survival

PFS	Progression Free Survival
PI	Principal Investigator
RR	Response Rate
SAE	Serious Adverse Event
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Advserse Reaction
UNL	Upper Normal Limit
US	Ultrasound
WBC	White Blood Cells
WHO	World Health Organization

4. INTRODUCTION AND BACKGROUND

4.1 Overview of current treatment options in Luminal, Estrogen Receptor-Positive, HER2 negative Breast Cancer

The presence of estrogen receptor (ER) is one of the most important predictive and prognostic markers in human breast cancer. Around 70% of all invasive breast cancers are hormone receptor (HR) positive at the time of diagnosis. [1] Consequently, anti-estrogen treatments that antagonize ER function or inhibit estrogen production (e.g. aromatase inhibitors [AIs]) have been extensively studied in breast cancer. [2,3] In the first-line metastatic setting, the use of non-steroidal AIs (Anastrozole, Letrozole) have shown superiority over tamoxifen and are considered standard HT therapy in post-menopausal women/ patients. [4,5] While Tamoxifen and/or ovarian function suppression (OFS) have significantly contributed to mortality reduction in premenopausal patients with early breast cancer (EBC), [6] a report has shown that the use of an AI (Exemestane) plus OFS may further offer a significant Progression-free survival (PFS) benefit compared to Tamoxifen plus OFS in young patients. [7] There are a limited number of randomized controlled trials related to endocrine therapy in premenopausal patients with metastatic breast cancer (MBC). In a meta-analysis of four studies on HR+ pre-menopausal MBC (n=506), the combination of luteinizing hormone-releasing hormone agonist (LHRHa) plus tamoxifen resulted in significantly prolonged progression-free survival (PFS) ($P < 0.001$) and OS ($P = 0.02$) compared to either agent alone. [8] As a result, OFS + HT is currently recommended as standard therapy in young women with MBC. [9] AIs alone are not indicated in premenopausal women but have shown efficacy in non-randomized phase II studies in combination with LHRHa. [10,11,12,13] In a prospective, single-arm, multicenter phase II trial from Japan, 32 premenopausal patients were treated with Goserelin and Anastrozole achieving a clinical benefit rate of 72%, and a median time to progression of 8.3 months similar to the results observed with AIs in postmenopausal women. [10] Other Phase II studies have confirmed the efficacy of AIs + OFS as first-line and second-line therapy of MBC. [11,12,13] In phase II parallel-group study, there was no difference in PFS between premenopausal patients receiving Letrozole plus Goserelin and postmenopausal patients treated with Letrozole alone (9.5 months and 8.9 months respectively). [13] Accordingly, the combination of OFS plus AIs may be considered as a therapeutic option in pre/ peri menopausal patients with MBC.

4.2. Overview of Palbociclib and role of CDK 4/6 Inhibitors

Palbociclib (PD 0332991) is the first-in-class CDK4/6 inhibitor that has shown a significant PFS advantage when combined with Letrozole in first-line therapy of MBC. [14]

Based on the efficacy and safety data from the PALOMA-1 trial, 2 phase III registration trials were performed in second-line therapy of MBC (PALOMA-3) [15] and first-line MBC (PALOMA-2) [16] comparing AIs versus AIs plus Palbociclib. Updated results of PALOMA-2 showed a significant improvement of Progression-Free Survival (PFS) in favor of the combination Letrozole plus Palbociclib over Letrozole (HR=0.56, CI, 0.46-0.69, p<0.000001). [17] A subgroup analysis confirmed similar efficacy in pre-menopausal patients. In terms of toxicity, Palbociclib and Letrozole combination was well tolerated, with uncomplicated neutropenia as the most frequent adverse event. [14] Consequently, the therapeutic index was favorable for the combination. Additionally, the phase III trial Monaleesa-7 comparing HT with or without Ribociclib (CDK 4/6 Inhibitor) in pre-menopausal patients confirmed the superiority of HT plus CDK 4/6 Inhibitor over HT in terms of PFS (HR= 0.55, CI, 0.44-0,69, p=0.000000098) and Overall Survival (OS), (HR=0.71, CI, 0.53-0.94, p=0.0097). [18] These results confirmed that HT plus CDK 4/6 inhibitors are considered today standard in first-line therapy of pre and post-menopausal patients with advanced luminal BC.

4.3 Laser Spectroscopy

The emergence of new infrared laser spectroscopic technology on liquid biopsy developed by the Max Planck Institute in Munich, Germany could open new perspectives. [19-23] The "Broadband Infrared Diagnostics (BIRD)" technique makes it possible to use a femtosecond molecular vibrational spectroscopy-type approach induced by controlled power lasers allowing to define "**molecular profiles at n Variables**" acquired by a simple method that is non-invasive, sensitive, low cost in the context of cancer screening. This infrared (IR) and near-infrared (NIR) laser spectroscopy technique rely on the scattering of photons as the incident light interacts with the target material. These interactions cause a frequency shift that reflects particular molecular vibration energy. These vibrations are correlated with a specific molecular bond that makes it possible to build a "biochemical/molecular imprint". Physiological or pathological changes lead to changes in the initial biochemistry and, consequently, changes in the spectra. These spectra are very detailed and integrated into a powerful computational approach that allows to detect "n variable specific molecular profiles'' that can be potentially correlated with plasma differences related to the presence or absence of cancers or through the alteration of combined compositions which could serve as diagnostic markers and prediction of cancer medication efficacy [24].

4.3.1 Technology

The first-generation BIRD technology, a broadband femtosecond infrared laser source, and an infrared wave sampling system for ultra-sensitive molecular vibration spectroscopy, and the design of a new Photon Fluorescence Multi-Microscope System, are operational in Munich at the Max Planck Institute/ Ludwig-Maximilians-Universität, München. [24]

This new performance regime of the technique is based on significant improvements in both the source and the detection of infrared radiation compared to the current state of this technique. The INFRALIGHT source presents a unique

combination of high power/brightness, wide bandwidth, and temporal coherence. High power/brightness is an essential prerequisite for achieving high detection sensitivity and short acquisition time.

In a noise-reduced, noise-free spectroscopic apparatus, such as time-resolved field sampling based on a femtosecond laser, the signal-to-noise ratio (S / N) is directly proportional to the coherent incident radiation power. Therefore, high power means an improved signal-to-noise ratio, which is very important for the detection of low concentration samples. Broad bandwidth is a prerequisite for recording almost complete files. In the case of complex organic mixtures consisting of hundreds of molecular species, this is crucial for the unequivocal identification of individual specimens.

The new source having dimensions of the order of the square meter will supplant considerably, in the two-way, advanced infrared technology synchrotron sources occupying areas of hundreds of square meters. The new source has significantly improved the acquisition sensitivity of infrared molecular fingerprints of cancer biomarkers in the blood.

4.3.2 Proof of Concept

The proof of concept of the value of this technology in cancer screening was obtained in the context of breast cancer following the quantum optics analysis of a series of liquid biopsies from 67 healthy controls (absence of breast cancer by standard screening) and 28 patients with breast cancer (King Saud University, Riyadh, Saudi Arabia). [25] Quantum Optics analysis was performed at the Max Planck Institute in Munich and the definition of "Molecular Profiles at n Variables" on the King Abdallah University for Science and Technology (KAUST) supercomputer in Thuwal, Saudi Arabia. The non-hierarchical data mining correlative analysis allowed to differentiate the molecular profiles between the 2 groups (controls versus patients) with a sensitivity of 97% and a specificity of 72% (11,380 variables). A second, more in-depth analysis achieved a sensitivity of 99% but with a slight decrease in specificity to 64%. An age-sensitive analysis found a sensitivity of 98% with a specificity of 97%, which appears extremely interesting and deserves to study the potential value of this approach in the context of the screening of other tumor types, as well as for the **prediction of efficacy of cancer medications, using the concept of singularity**.

5. ICRG0201 STUDY RATIONALE

There is strong evidence to suggest that the dual inhibition of CDK 4/6 and ER signaling is a highly effective therapeutic strategy in HR+ advanced BC and represents a new standard in 1st line therapy of advanced BC for pre/ peri and post-menopausal patients with luminal HER2 negative tumors. With the results of Palbociclib in the PALOMA trials' series, the combination of AI plus Palbociclib is now established as a standard in this setting (PALOMA-2). [16] While all subgroups of patients seem to benefit from the combination of a probabilistic approach, a major problem rests on the upfront identification of efficacy for individual patients.

This study will be the first program exploring the adjunction of the Quantum Optics technology on liquid biopsies to define individual 'molecular fingerprinting profiles' to predict the individual therapeutic effects of Palbociclib combined with Aromatase Inhibitors (AI) (plus OFS for pre/peri-menopausal patients) in Luminal, hormone receptor-positive and HER2 negative advanced breast cancer. Batteries of algorithmic tests will integrate the thousands of variables obtained

by Quantum Optics and will correlate to the 'individual molecular profiles at n variables' to a binary question (efficacy or not of the combination of Palbociclib + Aromatase Inhibitors (AI)). This approach is introducing the concept of singularity, in an attempt to break from the classic concept ‘one size fits all’.

6. STUDY OBJECTIVES

Primary Objectives

To assess the rate of objective clinical response, according to the revised RECIST guidelines for the combination of Palbociclib + Aromatase Inhibitors (AI).

To evaluate the value of infrared laser spectroscopy analysis on liquid biopsies in terms of prediction of the efficacy/resistance of the combination (Palbociclib + Aromatase Inhibitors (AI)) on an individual basis.

Secondary Objectives

- To evaluate Progression-Free Survival (PFS).
- To assess clinical benefit (CR/PR/Stabilization>24 weeks);
- To assess the ‘objective’ clinical benefit (CR/PR/Minor response [> 0%] > 24 weeks).
- To evaluate the modification of the individual molecular fingerprinting during the course of the treatment. To investigate the role of individualized molecular fingerprinting to predict early progression (>6 months).
- To investigate the role of individualized molecular fingerprinting to predict progression in age-matched patients with an initial response.
- To evaluate safety and tolerability.
- To perform Benchmark analysis, using Raman Spectroscopy.

7. STUDY DESIGN

This is a multicenter, open-label phase II study to assess the rate of objective clinical response, according to the revised RECIST guidelines for the combination of Palbociclib + Aromatase Inhibitors (AI) in women with luminal, HER2 negative Stage IIIb/IV Breast Cancer.

Eligible pre/peri-menopause* and post-menopausal patients, based on inclusion/exclusion criteria, will be prospectively treated with a combination of: Palbociclib (125 mg daily per os (3 weeks on-1 week off) with dose adaptation according to safety profile) and non-steroidal aromatase inhibitor- Letrozole (2.5mg) or Anastrozole (1mg) daily per os. This combination will continue until progression.

Treatment response will be evaluated every 3 months using clinical and radiological assessments (Revised RECIST guidelines).

Patients will undergo liquid biopsies for plasma molecular fingerprinting by the Quantum Optics technology at baseline (before start of trial treatment), after each treatment cycle for the first three months and then every 3 months until progression, along with clinical and radiological assessments every 3 months.

After documented progression, patients will be treated at Principal Investigator (PI)'s discretion.

*With oophorectomy for pre/peri-menopausal patients (surgery or Analogs of LHRH).

8. STUDY POPULATION

Women with luminal, HER2 negative Stage IIIb/IV Breast Cancer will be enrolled in the trial.

8.1 Number of Patients

Eighty (80) patients will be enrolled according to eligibility criteria.

8.2 Inclusion Criteria

To be enrolled in the study, patients should meet the following inclusion criteria:

1. Written informed consent before beginning specific protocol procedures including expected cooperation of the patients for the treatment and follow-up must be obtained and documented according to the local regulatory requirements.
2. Postmenopausal women or pre/peri-menopausal womenwith Surgical oophorectomy (preferred) or Analogs of LHRH.
3. Performance status < 3 (according to WHO criteria).
4. Histologically confirmed breast cancer (Luminal A or B).
5. Estrogen Receptor positive (ER > 1%).
6. HER2 negative (score 0 or 1 by immunochemistry), FISH negative if IHC score 2.
7. Clinical stage IIIb & IV.
8. Either:
    a. Women with De novo advanced luminal HER2 negative advanced breast cancer without other prior systemic treatment for advanced disease.
    b. Women with luminal HER2 negative advanced breast cancer either with secondary resistance (relapse after 2 years of adjuvant hormone therapy or within 12 months of completion of adjuvant HT) or sensitivity to adjuvant HT (relapse > 12 months after completion of adjuvant HT).
9. Measurable or evaluable disease.
10. Hematology:
    - Neutrophil count ≥ 1.5 G/L,
    - Platelet count ≥ 100 G/L,
    - Leucocyte count > 3.0 G/L,
    - Hb> 9g/dl.

11. Hepatic function:
    - Total bilirubin ≤ 1.5 times the upper normal limit (UNL),
    - ASAT ≤ 2.5xUNL,
    - ALAT ≤ 2.5xUNL,
    - Alkaline phosphatase ≤ 2.5 times the upper normal limit (UNL).
12. Renal function:
    - Serum creatinine ≤1.5xUNL (and if Serum creatinine >1.5xUNL, creatinine clearance ≥40 mL/min),
13. Metabolic function:
    - Serum calcium ≥ lower limit of normal.
14. Negative pregnancy test (urine or serum) within 7 days before registrationfor all women of childbearing potential. Patients of childbearing potential must implement adequate non-hormonal contraceptive measures during study treatment.
15. Patients with negative Human Immunodeficiency Virus (HIV) and/or Hepatitis B and/or Hepatitis C results.

8.3 Exclusion Criteria

To be enrolled in the study, patients should meet the following exclusion criteria:

1. Male patients.
2. HER2 positive tumors or unknown HR/HER2 status.
3. Triple-negative Breast Cancer (ER<1%).
4. Pregnant or breast-feeding women, or those who plan to become pregnant within 6 months post-treatment.
5. No willingness to use highly effective methods of contraception (per institutional standard) during treatment and for 6 months post-treatment.
6. Any form of breast cancer other than those described in the inclusion criteria, particularly inflammatory and/or loco-regional disease (stages I, II & IIIa).
7. Non-evaluable tumor.
8. Bilateral breast cancer.
9. Patients with a history of other cancer, except *in situ* cervical cancer or baso-cellular skin cancer, considered cured.
10. Patient has another disease, which is deemed incompatible with the inclusion in the protocol.
11. Heart, kidney, medullary, respiratory or liver failure.
    - Clinically significant cardiovascular disease (including myocardial infarction, unstable angina, symptomatic congestive heart failure, serious uncontrolled cardiac arrhythmia) at baseline.
    - History of interstitial lung disease e.g. pneumonitis or pulmonary fibrosis or evidence of interstitial lung disease at baseline,
    - Acute urinary infection, ongoing hemorrhagic cystitis at baseline.
12. Uncontrolled diabetes.
13. Symptomatic or progressive disorder of the central nervous system (CNS) at baseline.
14. Patients with positive Human Immunodeficiency Virus (HIV) and/or Hepatitis B and/or Hepatitis C results.
15. Significant psychiatric abnormalities.
16. History of hypersensitivity to studied treatment or excipients.
17. Known previous or ongoing abuse of narcotic drug, other medication or alcohol.
18. Any investigational agent within 30 days before initiation of study treatment.
19. Patient unwilling or unable to comply with study requirements.

9. STUDY CONDUCT

Potential patients will be screened for the eligibility criteria and once confirmed, will be enrolled in the trial within one month of confirmation of luminal, HER2 negative Stage IIIB/IV Breast Cancer. Prior to performing any study activities/evaluations, the potential patient must be thoroughly informed about all aspects of the study, including scheduled study visits and procedures, and must sign the informed consent form. A signed copy of the informed consent form should be given to the patient.

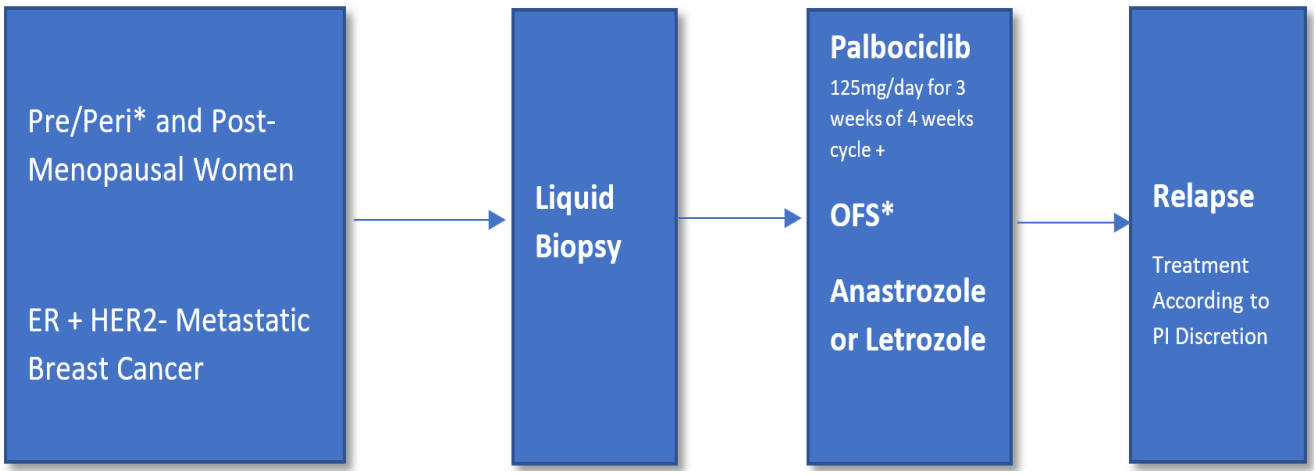
The registration form will then be immediately sent to the ICRG Medical Director before start of trial treatment.

Upon confirmation from ICRG Medical Director, a study specific subject number/ pseudonym will be allocated and the patient will then receive combination of Palbociclib (125 mg daily per os [3 weeks on-1 week off] with dose adaptation according to safety profile) and non-steroidal aromatase inhibitor: Letrozole (2.5mg) or Anastrozole (1mg) daily per os). The pre/peri menopausal patients will either have surgical oophorectomy or receive analogs of LHRH (e.g. Goseriline 3.6 mg subcutaneously every 4 weeks). This combination will continue until progression.

Patients will undergo liquid biopsies for plasma molecular fingerprinting by the Quantum Optics technology at baseline (before trial treatment), after each treatment cycle for the first three months and then every 3 months until progression along with clinical and radiological assessments every 3 months.

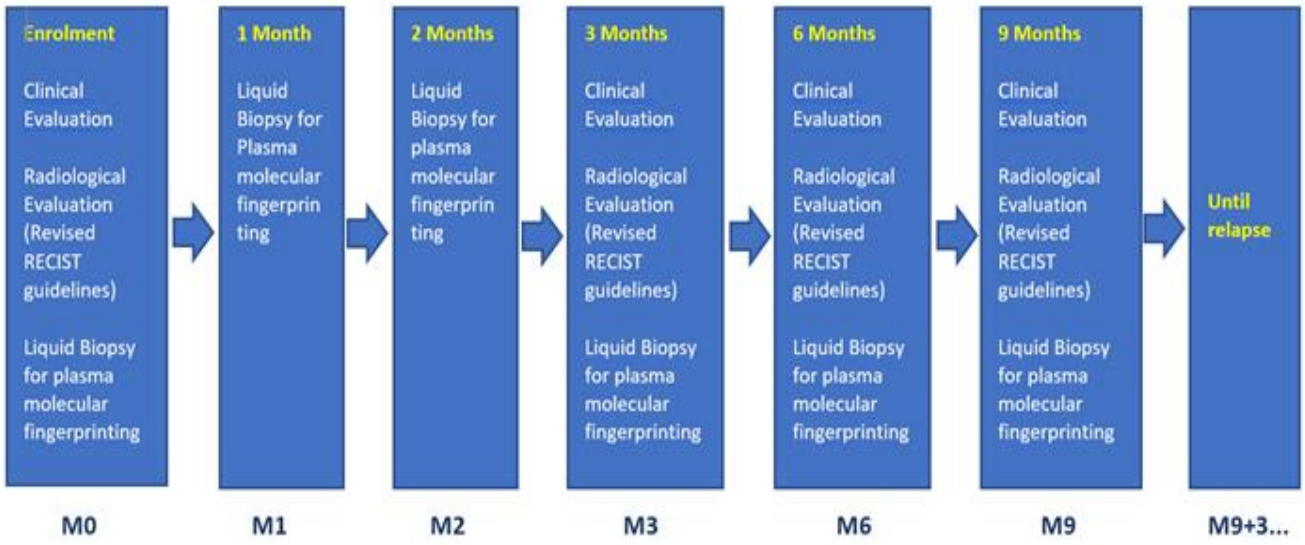
The samples will be shipped to Germany for 'BIRD' analysis only for participants with negative test results for Hepatitis B, Hepatitis C and Human Immunodeficiency Virus (HIV).

After documented progression, patients will end the study and will be treated at Principal Investigator (PI)'s discretion.



OFS after Anastrozole 1mg daily et Letrozole 2.5mg daily

8.1 Visit Schedule and Assessments



See also Appendix 1, 2 & 3 for Assessments, Liquid Biopsy and SOPs.

8.1. Scheduled Visits

8.1.1. Screening Visit/ Baseline Evaluation (D-28 to M0)

The following tasks will be performed at baseline (See Appendix 1):

- Informed consent review and signature before any study-specific activities/ evaluations. A signed copy of the informed consent should be given to the patient.
- Inclusion and exclusion criteria assessment.
- Registration after validation from ICRG Medical Director- All eligible patients must be registered with the International Cancer Research Group before the start of treatment. Official email with completed Registration Request Form to be sent to **ICRG Medical Director** for validation. Once approved, a trial specific subject number/ pseudonym will be allocated to each patient.
- Recording of demographic data & medical history, Prior/Concomitant medications.
- Confirmation of breast adenocarcinoma diagnosis (pathology report mandatory).
- Complete Physical Examination: Vital signs, height, weight, ECOG performance, and clinical evaluation.
- Mammography and/or Breast US if indicated.
- MRI if indicated and available.

- Radiological examinination (within 4 weeks before screening): Chest CT scan or X-ray &/or Abdominal/Pelvis CT scan or US &/or Bone scan.
- Bone Dexascan (osteodensitometry) within 4 weeks before or after enrollment, if indicated.
- Radiological evaluation with revised RECIST guidelines.
- Pregnancy test (urine or serum) only for patients with childbearing potential (within 7 days before enrollment).
- ECG (in case of use of Letrozole).
- Hematology: Hemoglobin, WBC and Neutrophil count, Platelet count (within 7 days before registration).
- Biochemistry:
    - Liver function: Alkaline Phosphatase, ASAT (SGOT), ALAT (SGPT), Bilirubin (within 7 days before registration; if **abnormal results**, liver function tests should be **repeated within 3 days** before registration).
    - Renal function: Serum Creatinine, (within 7 days before registration).
    - Metabolic function: Serum Calcium, (within 7 days before registration).
- Tumor Markers: CA15-3 and CEA are optional.
- Hepatitis B, Hepatitis C and Human Immunodeficiency Virus (HIV) test results (window of plus/ minus one month from the study visit) are required for all participants.
- Liquid biopsies for plasma molecular fingerprinting as per the trial SOP. Please refer to appendix 3.
- For pre/peri-menopausal patients: Analogs of LHRH (as indicated).

8.1.2. Trial Treatment Initiation (M0)

- Post-menopausal patients (including pre/peri menopausal patients with surgical oophorectomy): Palbociclib 125 mg daily per os (3 weeks on-1 week off) & Letrozole (2.5mg) OR Anastrozole (1mg) daily per os.
- For pre/peri-menopausal patients: Analogs of LHRH (as indicated) and Palbociclib 125 mg daily per os (3 weeks on-1 week off) & Letrozole {2.5mg} OR Anastrozole {1mg} daily per os.

8.1.3. Routine Visits (every 28 days)- M1 & M2, M3:

- Physical examination.
- Liquid biopsies for plasma molecular fingerprinting (Baseline, M1, M2, M3, and then every 3 monthly). These are mandatory at each of these visits before dispensation of trial medication.
- Hematology: Hemoglobin, WBC and Neutrophil count, Platelet count.

- Biochemistry: Liver function [Alkaline Phosphatase, ASAT (SGOT), ALAT (SGPT), Bilirubin], Renal function [Serum Creatinine] & Metabolic function [Serum Calcium].
- Adverse events (AEs) .
- ECG (in case of use of Letrozole).
- Drug accountability for drugs dispensed at the previous visit.
- Palbociclib {125 mg daily per os (3 weeks on-1 week off)} & Letrozole {2.5mg} OR Anastrozole {1mg} daily per os dispensed.
- For pre/peri-menopausal patients: Analogs of LHRH as indicated.
- Patient diary dispensed at M1 and reviewed at every visit.

8.1.4. Routine Visits (every 28 days)- M4, M5, M6, M7 onwards until progression:

- Physical examination.
- Hematology: Hemoglobin, WBC and Neutrophil count, Platelet count.
- Biochemistry: Liver function [Alkaline Phosphatase, ASAT (SGOT), ALAT (SGPT), Bilirubin], Renal function [Serum Creatinine] & Metabolic function [Serum Calcium].
- ECG (in case of use of Letrozole)- as indicated.
- Adverse events (AEs).
- Drug accountability for drugs dispensed at the previous visit.
- Palbociclib {125 mg daily per os (3 weeks on-1 week off)} & Letrozole {2.5mg} OR Anastrozole {1mg} daily per os dispensed.
- For pre/peri-menopausal patients: Analogs of LHRH as indicated.
- Patient diary reviewed.

8.1.5 Response assessment visits (every 12 weeks)- M3, M6, M9, M12 onwards until progression:

- Clinical evaluation.
- Radiology: Chest CT scan or X-ray &/or Abdominal/Pelvis CT scan or US &/or Bone scan. (Same as at baseline.)
- Radiological evaluation with revised RECIST guidelines.
- Liquid biopsies for plasma molecular fingerprinting every 12 weeks post the first 3 months' phase. These are mandatory at each of these visits before dispensation of trial medication.

8.1.6 End of the study visit

The end of study is defined as the day of confirmed progression. Drug accountability will be done for drugs dispensed at the previous visit. Adverse event data will be collected at this time.

10. MEDICAL PRODUCTS

10.1. Route and Dosage Form

- Palbociclib 125 mg daily per os (3 weeks on-1 week off) with dose adaptation according to safety profile.
- Letrozole 2.5mg or Anastrozole 1mg daily per os.

10.2. Treatment regimens and pre-medications

- Surgical oophorectomy (preferred) or Analogs of LHRH (e.g. Goserelin 3.6 mg every 28 days) for pre/peri-menopause patients.
- Duration & dosage of treatment will be adapted in case of toxicity.
- Standard prevention/treatment of osteopenia/osteoporosis is recommended as per standard clinical practice.

10.3. Early Treatment Discontinuation

Every attempt should be made to obtain the reason for early treatment discontinuation. The reason for the discontinuation of therapy will be documented on the CRF.

If a patient is withdrawn because of an adverse event, data of the Adverse Event should be collected as well. The patient will be followed according to the standard medical practice until the condition returns to normal or is considered stable or chronic unless the sponsor and the investigator agree to not further pursue the adverse event.

If there are multiple reasons for early discontinuation, the worst-case scenario should be chosen.

10.3.1. Criteria for Early Treatment Discontinuation

- A patient may withdraw or be withdrawn from the study for the following reasons:
- Patient withdrew consent
- Lost to follow-up/failure to return
- Adverse Event (specify primary AE in the AE log)

- Pregnancy
- Death
- Investigator's decision
- Sponsor's decision
- Non compliance with protocol
- Other

11. STUDY TREATMENTS

11.1. Palbociclib

Palbociclib will be provided by the ICRG for all trial patients.

11.1.1. Posology and method of administration

Palbociclib will be supplied by the ICRG as capsules containing 125 mg, or 100 mg, or 75 mg, and will be provided to eligible patients.

A patient diary will be used. Patients will be required to return all bottles & packs of study medications as well as the completed patient diary on Day 1 to D21 of each cycle for the drug accountability.

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

11.1.2. Dose interruptions and delays

The following adverse events will result in Palbociclib interruption and delay until retreatment criteria are fulfilled:

- Grade 4 neutropenia (ANC< 500/ mm^3).
- Grade 3 or 4 neutropenia (ANC<1000/mm^3) associated with a documented infection or fever 38.5°C.
- Grade 4 thrombocytopenia (Platelet < 25,000/ mm^3).
- Grade 3 Non-hematologic toxicity (including, nausea, vomiting, diarrhea, and hypertension only persisting despite optimal medical treatment).
- Concurrent > 3x ULN ALT and 2x ULN Total Bilirubin. If those occur, the dose needs to be held while the cause is being investigated.

11.1.3. Retreatment Criteria following treatment interruption for adverse event (AE) or at the beginning of a new cycle will be as follows:

- Platelet count ≥ 50,000/mm^3.
- ANC ≥ 1000/mm^3 and no fever.
- Grade ≥3 non-hematologic AEs (including nausea, vomiting, diarrhea, and hypertension only if persisting despite optimal medical treatment), have recovered to Grade ≤1 or baseline.
- In case the delay is related to hematologic toxicity, the frequency of blood count assessments will be adjusted as clinically indicated.

11.1.4. Dose reductions

A dose reduction of Palbociclib by one level is recommended depending on the type and severity of the toxicity as per Palbociclib's (Ibrance) Prescribing Information (See paragraphs 11.1.5 and 11.1.6).

All dose modifications/ adjustments will be documented in the patient's source and the CRF.

Table: Palbociclib Recommended Dose Modification for Adverse Event

Dose level	**Dose**
Recommended starting dose	125 mg/day
First dose reduction	100mg/day
Second dose reduction	75 mg/day*

* If further dose reduction below 75 mg/day is required, Palbociclib will be discontinued.

11.1.5. Dose Modification and Management a – Hematologic Toxicities

CTCAE Grade	**Dose Modifications**
Grade 1 or 2	No dose adjustment is required
ıde 3 [b]	No dose adjustment is required Consider repeating complete blood count monitoring 1 week later Withhold initiation of the next cycle until recovery to Grade ≤2
Grade 3 ANC (<1000–500/mm^3) + fever ≥38.5°C and/or infection	Withhold Palbociclib and initiation of the next cycle until recovery to Grade ≤2 (≥1000/mm^3) Resume at next lower dose

Grade 4 [b]	Withhold Palbociclib and initiation of the next cycle until recovery to Grade ≤2 Resume at next lower dose

Grading according to CTCAE Version 4.0.
ANC=absolute neutrophil count; CTCAE=Common Terminology Criteria for Adverse Events.
[a] Monitor complete blood count prior to the start of IBRANCE therapy and at the beginning of each cycle, as well as on Day 14 of the first two cycles, and as clinically indicated.
[b] Except lymphopenia (unless associated with clinical events, e.g., opportunistic infections).

11.1.6. Dose modification and management – non-hematologic toxicities

CTCAE Grade	Dose modifications
Grade 1 or 2	No dose adjustment required
Grade ≥ 3	Withhold treatment until symptoms resolution to: ● Grade ≤ 1 ● Grade ≤ 2 (if not considered a safety risk for the patient Resume at the next lower dose.

11.2. Letrozole & Anastrozole

Treatment with Letrozole or Anastrozole should be provided by the hospital and will be administered orally at the usual doses (Letrozole: 2.5 mg per day / Anastrozole: 1 mg per day). Management of side effects will follow the local practice.

For Letrozole QTc > 500 msec and potentially reversible causes (e.g., electrolyte imbalance, concomitant medications known to prolong QTc) corrected. If QTc remains above 480 msec, treatment may re-start but a cardiologist should be consulted, and the ECG monitored more frequently as per the investigator's best medical judgment until QTc < 480 msec.

11.3. Analogs of LHRH (Surgical oophorectomy preferred)

Treatment with analogs of LHRH (e.g. Goseriline 3.6 mg subcutaneously every 4 weeks) should be provided by the hospital and will be administered as per local practice for all pre/peri-menopausal women at study entry. Patients must have commenced treatment with LHRH agonist at least 4 weeks before the first dose of study treatment.

12. STUDY PROCEDURES

12.1. Liquid biopsy sampling

Infrared laser spectroscopy analysis will use a highly sensitive, non-invasive, non-marking molecular fingerprint based on femtosecond infrared laser spectroscopy, called BIRD: Broadband Infrared Diagnostics. The blood samples, collected

and stored in each participating centers before being prepared and shipped by batches to LMU/MPQ and KAUST for analysis using quantum optics analysis and Raman spectroscopy respectively according to the following steps:

- Samples will be collected at each visit (Baseline, M1, M2, M3, and every 3 months thereafter until progression).
- Sampling kits will be provided including EDTA K3 plasma tubes (4.9 ml) and Safety Multifly (Butterfly) needles (0.8 mm in diameter). A total of 20 ml/ will be collected for each liquid biopsy).
- Samples will be incubated at room temperature until the blood coagulates (at least for 20 minutes). The tubes will be then centrifuged within three hours for 10 minutes at 2000 g at room temperature. Then the supernatants will be aliquoted into 0.75 Micronic tubes (provided by ICRG) and deep-frozen at -80 °C for storage within two hours from aliquoting.
- No freeze-unfreeze cycle will be allowed.
- At least 6 aliquots of each plasma samples at each sampling time, for the possibility of carrying out several independent experimental measurements.
- SOP- Appendix 3 has been provided with the details of the procedure.

13. STUDY ENDPOINTS

13.1. Primary Endpoint

Rate of objective clinical response, according to the RECIST revised criteria for the combination of Palbociclib + Aromatase Inhibitors (AI).

Sensitivity and specificity of infrared laser spectroscopy analysis on liquid biopsies to predict the efficacy/resistance of the combination (Palbociclib + Aromatase Inhibitors (AI)) on an individual basis.

13.2. Secondary Endpoints

- Progression-Free Survival (PFS).
- Clinical benefit (CR/PR/Stabilization>24 weeks).
- 'Objective' clinical benefit (CR/PR/Minor response [> 0%] > 24 weeks).
- Modification of the individual molecular fingerprinting during the course of the treatment.
- Modification of individualized molecular fingerprinting to predict progression in patients with initial response and objective clinical benefit.
- Modification of individualized molecular fingerprinting to predict progression in age-matched patients with initial response and objective clinical benefit.
- Modification of individualized molecular fingerprinting to predict early progression (>6 months).
- Safety and tolerability.
- Modification of individualized molecular fingerprinting to predict progression obtained from Raman spectroscopy as a benchmark analysis.

13.3. Safety Endpoints

- Incidence and frequency of adverse events, serious adverse events.

- Proportion of patients (%) who prematurely discontinued study treatment.
- Proportion of patients (%) who prematurely discontinued study treatment due to AEs.

14. SAMPLE SIZE CONSIDERATION

The primary objectives of this trial are:

- to evaluate the rate of objective clinical response, according to the revised RECIST guidelines for patients treated with a combination of Palbociclib + Aromatase Inhibitors (AI)and;
- to evaluate the value of infrared laser spectroscopy analysis on liquid biopsies in terms of prediction of the efficacy of the combination (Palbociclib + Aromatase Inhibitors (AI)) on an individual basis.

Considering the response rates (RR) reported in PALOMA 2 trial (overall response rate/ORR: 42% and ORR for measurable disease: 55%), we expect a 50% objective Response Rate.

Considering the initial published data of infrared laser spectroscopy analysis on liquid biopsies comparing plasma molecular fingerprint profiles between a control population without breast cancer and a population with breast cancer with a sensitivity of 98% and a specificity of 97%, we hypothesize that the probability of response to Palbociclib + Aromatase Inhibitors (AI) will be 50% and the sensitivity to differentiate responders to non-responders by infrared laser spectroscopy analysis on liquid biopsies (prediction of efficacy) will have a sensitivity of 97%.

Based on the above hypothesis, the sample size calculated using alpha 5% and power 95%, is N=80 patients to be enrolled and treated.

15. STATISTICAL METHODS & DATA ANALYSIS

15.1. Populations:

- The Intent-to-Treat (ITT) population will consist of all patients enrolled in the study and treated with combination Palbociclib + Aromatase Inhibitors (AI).
- The Full-Analysis (FA) population will consist of all patients enrolled in the study and treated with combination Palbociclib + Aromatase Inhibitors (AI), without major protocol deviations.
- The safety population will consist of all patients who received any study drug and had at least one post-baseline safety assessment.

15.2. Statistical Methodology

Qualitative variables will be described by frequency and percentage. A comparison of the proportion between the two groups of responders (responders and non-responders) will be made using a chi-square test. The 95% confidence intervals of the different response rates will be given.

Quantitative variables will be described by the mean, standard deviation, median, maximum and minimum.

Time-dependent parameters will be computed by the Kaplan-Meier method to take censured data into account. The Kaplan-Meier product-limit method will be used to estimate the PFS and OS.

Cox's proportional hazards regression analysis will be performed for response rates, clinical benefits, PFS.

All tests of hypotheses will be one-sided. Confidence intervals of the median survival will be calculated using the method of Simon.

Demographic data and baseline characteristics will be presented for the ITT population.
The analysis of efficacy endpoints will be performed on the FA population. The safety analysis will be performed using the safety population. Patients with major protocol deviations will also be described.

15.3. The methodology of computational analysis of "biological profiles at n variables"

15.3.1. Batteries of algorithmic tests definition:

We propose a "non-hierarchical data mining" approach with:

- An analysis with the support vector machines with a polynomial nucleus, which will give, during the analysis of treatment responders/non-responders, highly precise analyses.
- Age stratifications when applicable.
- Presence of sub-stratifications in different age groups.

This study will be the first program exploring the adjunction of the Quantum Optics technology on liquid biopsies to define individual ‘molecular fingerprinting profiles’ to predict the individual therapeutic effects of Palbociclib combined with Aromatase Inhibitors (AI) (plus OFS for pre/peri-menopausal patients) in Luminal, hormone receptor-positive and HER2 negative advanced BC. Batteries of algorithmic tests will integrate the thousands of variables obtained by Quantum Optics and will correlate the 'individual molecular profiles at n variables' to a binary question (efficacy or not of the combination of Palbociclib and Aromatase Inhibitors (AI)). This approach is introducing the concept of singularity, breaking from the classic concept ‘one size fits all’.

16. SAFETY PARAMETERS

16.1. Adverse Events (AEs)

Adverse events will be recorded from when a patient has signed the Informed Consent Form (ICF) and throughout the study until end of study visit. They should be reviewed and updated at the end of each study visit.

The severity of the toxicities will be graded according to the NCI CTCAE v5.0 whenever possible. See https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

16.1.1 General definition

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. Any variation of the studied disease, except for serious impairment, is not considered an adverse event. Any event occurring during the trial (from ICF signature to end of study visit) must be reported in the case report form.

As far as possible, each AE should be evaluated to determine:

1. The severity grade (CTCAE grade 1-5).
2. The relationship to study drugs (suspected/not suspected)

   The investigator must do his/her best to explain each adverse event and establish when it exists, the connection with each trial's product.

   Causality will be established for each product as follows:

   - no, it is not related;
   - yes, there is a reasonable possibility of causality according to the following criteria:
     - the pharmacology of the product is known,
     - the effects are similar compared to already known effects reported for this product or other products of the same family or category of the compound,
     - the adverse event already reported in the literature for similar products and is considered as related to the study product,
     - adverse event tightly related to the treatment period (between the beginning of the administration – the drug challenge period – and the end of administration – de-challenge period) or positive re-challenge.
3. The duration (start and end dates or if continuing at the final exam).
4. The actions are taken (no action is taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication is taken; non-drug therapy given; hospitalization/prolonged hospitalization).
5. The seriousness of the Adverse Events (AEs) in keeping with the definition of Serious Adverse Events (SAE), defined in section 15.2.2.

16.1.2. Adverse Events follow-up

All AEs, serious and non-serious, occurring during the course of the clinical trial (i.e., from signing the informed consent onwards until end of study), regardless of relatedness or listedness, will be collected, documented and reported to the sponsor by the investigator on the appropriate reporting forms.

All AEs, including those persisting after the end of study treatment, must be followed up until they have resolved or have been sufficiently characterized unless the sponsor and the investigator agree to not further pursue them.

16.1.3. Known undesirable effects of study treatments

The known undesirable effects of study treatments are described in the Investigator's brochures and Summary of Product Characteristics.

16.2. Serious Adverse Events (SAEs)

16.2.1. General definition

A serious adverse event is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- induces a congenital anomaly/birth defect or abortion,
- is medically relevant.

The terms disability and incapacity match with all physical/psychological temporary or permanent handicap, clinically significant with consequences on the physical activities and/or the quality of life of the patient.

Important medical events are those which may not be immediately life-threatening but may jeopardize the patient and may require intervention to prevent one of the other serious outcomes listed above. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations such as important medical events (clinical or biological events) that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; the occurrence of second cancer; pregnancy; or development of drug dependency or drug abuse.

Inpatient **hospitalization** or prolongation of existing hospitalization means that hospital inpatient admission and/or prolongation of hospital stay were required for treatment of AE, or that they occurred as a consequence of the event. It does not refer to pre-planned elective hospital admission for treatment of a pre-existing condition that has not significantly worsened, or to the diagnostic procedure.

The term **"life-threatening**" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. The following events are not considered serious adverse events:

- a hospitalization < 24 hours,
- or a hospitalization planned prior entry into the trial,
- or a hospitalization scheduled by the protocol (for pan endoscopy, surgery…),
- or a hospitalization related to the tumor progression of the Breast Cancer,
- a tumor progression of the Breast Cancer,
- or a death related to the progression of the Breast Cancer.

16.2.2. Definition of an unexpected serious adverse event (U-SAE)

The event is expected when it is mentioned in the most recent version of the Investigator's Brochure or the Summary of Products Characteristics (SPC) for the product or is included in the Adverse Reaction of the relevant Reference Safety Information by its specificity, severity, outcome, and frequency.

16.2.3. Definition of a suspected unexpected serious adverse event (SUSAR)

A SUSAR is a serious adverse event that is mentioned neither in the Investigator's Brochure nor in the Summary of Product Characteristics (SPC) for drugs, or differing from the Brochure's or SPC' description in its nature, intensity, outcome or frequency.

The reference document to assess expectedness for the different study drugs is a Summary of Product Characteristics (SPC).

16.2.3.1. Intensity Criteria

The intensity criteria must not be confused with the seriousness criterion that is used to define the obligations of the declaration. The event intensity is to be estimated according to the CTC-AE version 5.0 classification.

The intensity of the adverse events not listed in this classification will be evaluated according to the following scale:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

16.3. Reporting Serious Adverse Event

To satisfy regulatory requirements, all Serious Adverse Event, whether deemed related to study treatment (Palbociclib OR Letrozole/ Anastrozole) or whether or not related to the research, which occurs **from the date of signature of the consent until end of study,** must be reported to the ICRG within 24 hours after the investigator has become aware of its occurrence. The SAE form completion and reporting must not be delayed even if all of the information is not available at the time of the initial contact.
The SAE should be submitted within 24 hours of becoming aware of the event to the ICRG.

Additional information (follow-up) about any SAE unavailable at the initial reporting should be forwarded by the site within 24 hours of the information becoming available to the ICRG.
Both initial and follow-up SAE reports will be sent to the ICRG Medical Director within 48 hours.

Second cancer, whether or not related to the research, must be reported to the ICRG Medical Director **up to the end of study**.

Notification must be carried out immediately and no later than 24 hours following knowledge, by business email to ICRG Medical Director by sending the form entitled “notification of a serious adverse event”, located in the investigator file, completed as precisely as possible, dated and signed by the investigator:
For each serious event, the investigator will report:

- Investigator Name and Center Number.
- Patient Number.

- Patient Demographics.
- Clinical Event
    - Exact Medical term
    - Description, as clearly as possible and per medical terminology,
    - Date of onset,
    - Severity,
    - If the protocol treatment was interrupted,
    - Relationship to study drug (causality),
    - Relationship to the study schedule or a protocol procedure (e.g. treatment scheme, additional examinations specifically requested by the protocol, etc.),
    - Relationship to the treated pathology, to another concomitant pathology, or another treatment,
    - Action was taken regarding the medicinal product,
    - Outcome: the event will be followed until remission or recovery of the baseline status or stabilization of possible sequelae.

Whenever possible, the investigator should also provide:

- Medical history case report form (copy).
- A copy of the hospitalization or prolonged hospitalization report.
- If the AE results in death:
    - Cause of death (whether or not the death was related to study drug),
    - Copy of the autopsy report (if available).
- A copy of all complementary examinations results (laboratory, discharge, X-Ray, etc.), including the relevant negative results (with normal laboratory values).
- Any other document (medical history, concomitants medications, etc.) that is relevant in the investigator's opinion.

All these documents must be made anonymous. More information might be requested by the ICRG Medical Director or the study CRA.

16.4. Serious Adverse Event Follow-up

Serious adverse events should be reported by the site to their local EC/IRB as dictated by their board's policies and procedures. ICRG will be responsible for submission to local regulatory authorities as per the country-specific guidelines.

Patients who have had an SAE during the study period must be followed clinically until all parameters (including laboratory) have either returned to normal or have stabilized or are otherwise explained.

The investigator is responsible for the appropriate medical follow-up of the patients until the recovery, stabilization or death of the patient. If required, follow-up could be maintained beyond the end of the trial period.. The investigator provides complementary information to the ICRG Medical Director using an SAE declaration form within 48 hours of its obtention. The last follow-up form is sent once recovery or stabilization is observed. The investigator keeps all the documents regarding the SAE to answer further information demands from the ICRG.

17. REGULATORY & ETHICAL ISSUES

17.1. Local Regulations/Declaration of Helsinki

This study will be conducted following the ethical principles that have their origin in the Declaration of Helsinki (2013) and that are consistent with "Good Clinical Practice" ICH Tripartite Guideline and the applicable laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.

17.2. Informed Consent

The principles of Informed Consent, according to the Declaration of Helsinki (2013) 1964 and the International Conference of Harmonization (ICH, E6 (R2)) step 4.8.8 guidelines on Good Clinical Practice (GCP), and the Local Regulations will be followed.

A patient is not allowed to enter a clinical study until she has been properly informed, has been given time to contemplate participation, and has freely given her consent by signing and dating the Ethics Committee/Institutional Review Board (EC/IRB) approved informed consent form. This must be done before performing any study-related procedures. **Information given to the trial participants** must cover all of the elements defined in the trial and must be written in a simple and comprehensible patient-appropriate manner.

The proposed consent form and any other documents relevant to the consent process must be submitted to the EC/IRB, together with the protocol, and must be approved before the study start.

A copy of the fully signed and dated Informed Consent Form and any other documents relevant to the consent process will be given to the patient and the original will be maintained at the site. **The consent forms** must be dated and signed by both the participant in research and the investigator. The original document is archived by the investigator; a copy is given to the research participant.

The information sheet and informed consent forms must be associated with the same document to ensure that the whole information is given to the participant.

17.3. Patient Confidentiality

All patient data will be identified only by a patient identification number, and date of birth. If dummy initials are used, the investigator should keep the dummy initials corresponding to the real patients' identification in the "Patient Identification Log".

After obtaining a patient's consent, the investigator will permit the study monitor, independent auditor or regulatory agency personnel to review that portion of the patient's medical record that is directly related to the study. This shall include all study relevant documentation including patient medical history to verify eligibility, laboratory tests result, admission/discharge summaries for hospital admissions occurring while the patient is on the study, and autopsy reports for deaths occurring during the study (if applicable).

17.4. Ethics Committee (EC) / Institutional Review Board (IRB)

The study must have unconditional approval in writing, by an appropriate Ethics Committee/Institutional Review Board (EC/IRB). A copy of the Letter of Approval from the EC/IRB, which contains specific identification of the documents approved, must be received by ICRG before site initiation.

Any amendments to the protocol or subsequent changes to the informed consent form as a result of changes to the protocol must also be approved by the EC/IRB and documentation of this approval provided to ICRG. Records of the EC/IRB review and approval of all documents about this study must be kept on file by the investigator and are subject to the sponsor's audit and/or regulatory authority inspection, during or after completion of the study.

Serious adverse events (SAEs) must also be reported to the EC/IRB by the investigator or the sponsor, according to local requirements.

Periodic status reports must be submitted to the EC/IRB as required, as well as notification of completion of the study and a final report where applicable. A copy of all reports submitted to the EC/IRB must be sent to the sponsor, where required.

17.5. Protocol Amendments

Changes to the protocol can only be made by an approved protocol amendment. Protocol amendments must be approved by the ICRG, Country regulatory authority, Central EC/IRB and/or each respective site's EC/IRB (as applicable), before implementation.

17.6. Liability and Insurance

Certificate of Clinical Trials Insurance will be provided by ICRG to the study sites.

18. DATA QUALITY ASSURANCE/SITE MONITORING

18.1. Quality Assurance

During the study, monitoring (remote and/or on-site) visits will be conducted at regular intervals. The monitoring visits will be conducted to ensure protocol adherence, quality of data, the accuracy of entries in the eCRF, drug accountability, compliance with regulatory requirements and continued adequacy of the investigational site and its facilities.

The site may be audited and/or monitored by a quality assurance officer named by the ICRG and/or regulatory authorities may wish to perform on-site audits. The investigator will be given notice before an audit occurs and will be expected to cooperate with any audit, provide assistance and documentation (including source data) as requested.

To guaranty the authenticity and credibility of the data as per the Good Clinical Practices, ICRG will set up an assurance quality program that includes:

- management of the trial according to specific operating procedures,
- quality control of the data provided by the investigator site is performed by the study monitor whose role is to match and check the consistency of the data reported in the eCRF concerning the source-documents,
- possible audit of investigational sites,
- centralized and statistical review of the selected criteria of the protocol.

18.2. Remote or on-Site Monitoring and Data Retention

Before study initiation, at a remote or on-site initiation visit, an ICRG representative will review the protocol and CRFs with the investigators and their staff. During the study, the field monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol and Good Clinical Practice, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigator must maintain source documents for each patient in the study, consisting of the case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information on eCRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient).

The investigator must give the monitor access to all relevant source documents to confirm their consistency with the eCRF entries. ICRG monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of data that will be used for all primary and safety variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan. No information in source documents about the identity of the patients will be disclosed.

All information on the eCRFs must be traceable to these source documents in the patient's file. Data not requiring a written or electronic record will be defined before the study start and will be recorded directly on the eCRFs, which will be documented as being the source data.

19. DATA COLLECTION AND VALIDATION

Patient data will be entered by site personnel using an eCRF, build on an electronic data capture (EDC) platform . This application will be set up for remote data entry. The EDC software has been fully validated and conforms to 21 CFR Part 11 requirements. Investigator site staff will not be given access to the EDC system until they have been fully trained by the ICRG representative. ICRG will supply the investigator site with guidelines for data entry into the eCRFs. Designated investigator staff will enter the data required by the protocol into the eCRFs using this web-based application. Automatic validation programs check for data discrepancies in the eCRFs and, by generating appropriate error messages, allow modification or verification of the entered data by the investigator staff before confirming the data. Queries are generated by the EDC system and designated investigator or site staff is required to respond to the query and make any necessary changes to the data on the eCRF.

The investigator must certify that the data are complete and accurate by applying an electronic signature to the eCRFs.

The data collected will be encoded and stored electronically in a database system.

Adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

At the end of the study, the occurrence of any protocol deviations will be determined. After these actions have been completed and the database has been declared to be complete and accurate, it will be locked. Any changes to the database after that time can only be made by a joint written agreement between the study statistician and ICRG.

20. INVESTIGATOR'S FILES & RETENTION OF DOCUMENTS

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These records include but are not limited to, the identity of all participating patients, all original signed informed consent documents, copies of all ECRFs, safety reporting forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence. These documents should be classified into two different separate categories: Investigator Study File and Patient Clinical Source Documents.
The records should be retained by the investigator according to International Conference on Harmonization (ICH), local regulations, or as specified in the Clinical Trial Agreement (CTA), whichever is longer.

Until the trial results are published, the investigator is responsible for ensuring **the confidentiality of the totality of the information**, handled by herself/himself and all other individuals involved in the course of the trial, that is supplied by ICRG. This obligation holds neither for the information that the investigator may communicate to the patients within the context of the trial nor for the already published information.
The investigator commits not to publish, not to spread or use in any manner, directly or indirectly, the scientific and technical information related to the trial.
Nevertheless, both the center and the investigator may communicate information relative to the trial:

- to the Health Minister,
- to the public health inspectors,
- to the regulatory agencies and inspectors.

The trial will not be the subject of any written note and/or oral comment without the prior agreement of the sponsor; the totality of the information that is communicated or obtained during the course of the trial belongs in full right to ICRG or otherwise specified, who can freely use it.

21. USE OF INFORMATION & PUBLICATION

All information supplied by ICRG in connection with this study and not previously published to the public is considered confidential information ("Confidential Information"). This Confidential Information includes the clinical protocol, case report forms, and other scientific data. Any data collected during the study are also considered Confidential Information. This Confidential Information shall remain the sole and exclusive property of ICRG, shall not be disclosed to others without the prior written consent of ICRG, and shall not be used except in the performance of this study.

ICRG has full ownership of the eCRF data completed as part of the study.

By signing the clinical study protocol, the investigator agrees that the results of the study may be used for publication, and information for medical and pharmaceutical professionals.

It is agreed that consistent with scientific standards, a publication of the results of the study shall be made only as part of a publication of the results obtained by all sites performing the protocol.

If ICRG and the study Chairman Prof. Jean-Marc Nabholtz chooses to publish the data from this study a copy will be provided to the investigator before the submission to the intended publisher. The publication authorship will include the chairman of the study, the presidents and principal investigators, participating investigators, ICRG/LMU members, and the funder's appointed member according to their active involvement in the trial.

In an equal manner, a publication of the sub-studies (biological studies) will require ICRG permission.

ICRG permission is required for publication but is reflective of applicable laws and regulations. The Publication Policy will be addressed in the Research and Financial agreement, and all details outlined in the agreement will apply to this protocol.

22. DECLARATION OF INVESTIGATOR

ICRG0201

Multicenter, first-line metastatic open-label prospective phase II trial evaluating the combination of Palbociclib (CDK 4/6 Inhibitor) and hormone therapy (Letrozole or Anastrozole) in women with luminal, HER2 negative advanced breast cancer: Evaluation of the prediction of individual treatment efficacy using infrared laser spectroscopy analysis on liquid biopsies.

I agree that the protocol contains all the details necessary to conduct the ICRG0201 study. I will conduct the study as described in this protocol and per Good Clinical Practice. I would do my best to recruit topics for this study and follow the guidelines of Good Clinical Practice during the patient consent process. As an investigator, I have guaranteed the proper conduct of the test as described in the protocol.

I agree that the collected patientdata is the property of ICRG as well as any other information from ICRG. The same is true of the results of the analyses of this study.

I agree that all source documents (consent forms, source documents with patient data, etc.) will be archived in the center with limited access to the staff involved in the study.

_____
Last Name/First Name of Investigator

_____
Date and Signature of Investigator

_____
Date and Signature of Medical Director (ICRG)

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24. APPENDICES

Appendix 1: Study Evaluations Flow Chart

	Initial assessment (between D-28 and D1)	**Before each cycle of Palbociclib**	**After 3 Cycles and Every 12 weeks thereafter until** progression
Informed Consent	✔		
Disease history	✔		
Physical examination	✔	✔	✔
Evaluation of toxicity		✔	✔
Pregnancy test (if indicated)	✔		
Blood count, platelets	✔	✔	✔
Blood liver, renal functions, calcium.	✔	✔	✔
CA 15-3 and CEA Optional	✔		
Clinical Evaluation	✔		✔
Radiologic Evaluation with RECIST Criteria. **CT Scan,** or equivalent, **Bone Scan,** according to investigator**s**	✔		✔
ECG (if indicated: Letrozole)	✔	✔ (as indicated)	

Appendix 2: Summary of biologic samplings for plasma molecular fingerprinting

	Before treatment	**At each cycle (every 4 weeks) for 12 weeks**	**After 3 Cycles: Every 12 weeks thereafter until progression.**
Blood samples for quantum optics (20 ml, EDTA K3 tubes 4.9 ml), centrifuged at 2g and 0,75 ml plasma aliquoted in at least 6 micronic tubes stored at -80°C.	✔	✔	✔

Appendix 3: SOP for liquid biopsies

Purpose:

The purpose of this SOP is to describe the process for assigning the patient identification number & sample identification number, blood sampling, preparation of plasma samples, and aliquotation of the samples into appropriate sample tubes and correct storage of the samples until transportation.

Scope:

This SOP applies to all site personnel involved in patient enrolment, & handling of blood samples for the ICRG0201 trial.

Definitions/Abbreviations:

Word / Terminology / Concept	Definitions and Abbreviations
eCRF	Electronic Case Report Forms
ICF	Informed Consent Form
ICRG	International Cancer Research Group
PI	Principal Investigator
SOP	Standard Operating Procedures

Procedure:

The pseudonym describes the encryption code between clinical data and the real name of a study patient . The pseudonym is noted in a **pseudonymization list** that is maintained at the site. This list is to be treated confidentially. The **Sample ID** describes the encryption code between blood samples and the pseudonym.

The pseudonyms each consist of a number of the type ICRG0201_000, where 000 describes an arbitrary number between 001 and 999.

A patient who signs the consent for the study will be assigned a specific patient **identification number** (pseudonym) and a sample-specific **sample identification numbers** (sample IDs). Preprinted **sample sheets with attached Sample IDs** are provided for this purpose by ICRG. The sample sheets consist of an A4 sheet with a blank line for the pseudonym & the printed Sample IDs.

1. This Sample ID is listed on the sample sheet and forms the core element for the blood collection tube and aliquot labels. For assigning the Sample ID at the time of blood collection, a new sample sheet is arbitrarily taken. On this sample sheet, the Pseudonym of the patient is noted in the box provided.
2. Before blood collection, **EDTA-K3 plasma tubes (4.9 ml Sarstedt Monovette 04.1931)**, and the required number of **0.75 micronic tubes (Safe Lock Tubes)** for plasma storage will be labeled.

a. From the sample sheet, the two bar codes with the 7-digit Sample ID are now taken and glued to the blood collection tubes. Two tubes of blood plasma will be collected + 2 tubes for storage (20mL collected and sampled in total).
b. The remaining ten 8-digit barcodes are glued to the plasma aliquots prepared in the laboratory.

3. The blood is collected using **Safety Multifly (Butterfly)** needles with a diameter of **21G/0.8 mm (Sarstedt order number 85.1638.235, color code: green).** Blood can also be collected through **an existing port or permanent venous catheter with a diameter of > 0.8 mm.**
   - ***Important***:
     - Only the above-mentioned needle diameter may be used since a change in the needle diameter can have a great influence on the laser measurement. If the cannulas are too fine, or when the punch is too tight, hemolysis may occur.
     - Care must be taken to ensure that the blood collection tubes are filled up to the max. fill level, so that sufficient plasma can be pipetted per patient .
     - After blood collection, slightly sway the blood collection tubes, but do not shake them.
4. For blood clotting, store the tubes standing at room temperature. The coagulation process takes **about 20 minutes**. The coagulation must be completed before centrifugation.
   - ***Important***: The period between blood sampling and centrifugation **should not exceed three hours**. If the three hours between blood sampling and centrifugation cannot be complied with, please inform the study team (see below for contact details).
5. The time of blood sampling and centrifugation is recorded on the sample sheet and transferred to the eCRF.
6. The tubes are then centrifuged at **2,000 g for 10 min at room temperature** (please note that the rpm will differ depending on the rotor size of the centrifuge).
   - ***Important***: Lower rotation speed will lead to an insufficient separation of solid and liquid blood components. Faster rotation speeds can lead to damage to the blood cells and thus to hemolysis.
7. The supernatant is then transferred into the pre-**labeled** tubes using a pipette **that is preset to** the 500 µl. The plasma obtained should be transferred in maximally 10 × 500µl aliquots, but at least in each case 3 × 500 µl aliquots. The number of aliquots should be documented in the pseudonymization list. If the minimum volume of 3 aliquots cannot be reached, please inform the ICRG team.
   - ***Important***:
     - Care must be taken that plasma is pipetted without disturbing the pellet.
     - Always use the orange tubes for aliquots from plasma samples (0.75ml pre capped Screw Tubes, 2D-coded with external thread, V-bottom incl. Screwcaps in orange).
     - The plasma tubes of a study participant should always be arranged in a row (left to right, A1 to H12), with no free space left between the different samples.
     - The samples of a study participant must not be stored in different cryo boxes. If necessary, a new cryo box may be started despite remaining spaces.
8. The micronic tubes are stored at **-80 ° C** in designated racks. The tubes must be stored at the latest two hours after centrifugation at - 80 ° C in the appropriate cryo boxes.
9. The samples must be transported at **-80 ° C**. A corresponding dry ice transport will be organized by the ICRG team.

Process Steps:

#	Description of Activities	Responsibility	Ref Docs
1	Patient consent was taken and eligibility is confirmed.	PI/ Co-PI	Protocol & ICF.
2	Pseudonym is assigned.	PI/ Co-PI or designee	
3	EDTA-K3 plasma tubes (4.9 ml Sarstedt Monovette 04.1931), and the required number of 0.75 micronic tubes (Safe Lock Tubes) for plasma storage are labeled.	Laboratory Technician	
4	Blood collection is done using Safety Multifly (Butterfly) needles with a diameter of 21G/0.8 mm (Sarstedt order number 85.1638.235, color code: green).	Laboratory Technician	
5	For blood clotting, the tubes are kept standing at room temperature.	Laboratory Technician	
6	The tubes are then centrifuged at **2,000 g for 10 min at room temperature.**	Laboratory Technician	
7	The supernatant is then transferred into the pre-**labeled** micronic tubes using a pipette **that is preset to** the 500 µl.	Laboratory Technician	
8	The micronic tubes are stored at **-80 ° C** in designated racks.	Laboratory Technician	
9	Samples are transported at **-80 ° C** once instructed by ICRG.	Site staff	

Appendix 4: Declaration of Helsinki

HELSINKI DECLARATION OF THE AMM - ETHICAL PRINCIPLES APPLICABLE TO MEDICAL RESEARCH INVOLVING HUMANS

Adopted by the 18th General Assembly AMM, Helsinki, Finland, June 1964 and amended by: 29th General Assembly AMM, Tokyo, Japan, October 1975
35th General Assembly AMM, Venice, Italy, October 1983 41th General Assembly AMM, Hong Kong, September 1989
48th General Assembly AMM, Somerset West, South Africa, October 1996 52th General Assembly AMM, Edinburgh, Scotland, October 2000
53e General Assembly AMM, Washington DC, USA, October 2002 (add a clarification note) 55e General Assembly AMM, Tokyo, Japan, October 2004 (add a clarification note)
59e General Assembly AMM, Seoul, South Korea, October 2008 64e General Assembly AMM, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Helsinki Declaration as a statement of ethical principles applicable to medical research involving humans, including research into human biological material and identifiable data.
2. The Declaration is conceived as an inseparable whole. Each paragraph should be applied taking into account all other relevant paragraphs.
3. In accordance with the mandate of the WMA, this Declaration is aimed primarily at doctors. The WMA, however, invites others involved in medical research involving humans to adopt these principles.

General principles

1. The Geneva Declaration of the AMM commits physicians in these terms: "The health of my patient will prevail over all other considerations" and the International Code of Medical Ethics states that "a physician must act in the best interest the patient when he is treating him ".
2. The duty of the physician is to promote and safeguard the health, well-being, and rights of patients, including those involved in medical research. The doctor devotes his knowledge and his consciousness to the fulfilment of this duty.
3. Medical progress is based on research that, ultimately, must involve human beings.
4. The primary purpose of medical research involving humans is to understand the causes, development, and effects of diseases and to improve preventive, diagnostic and therapeutic interventions (methods, procedures, and treatments). Even the best-proven interventions need to be continually evaluated through research into their safety, effectiveness, relevance, accessibility, and quality.
5. Medical research is subject to ethical standards that promote and ensure respect for all human beings and protect their health and rights.
6. If the primary purpose of medical research is to generate new knowledge, this goal should never override the rights and interests of those involved in the research.
7. It is the duty of physicians engaged in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy and confidentiality of the information of those involved in the research. The responsibility to protect those involved in this research must be held by a physician or other health professional, and never with those involved in the research even if they have given their consent.
8. In medical research involving human beings, physicians must take into account the ethical, legal and regulatory

standards and standards applicable in their own country as well as international norms and standards. The protections guaranteed by this Declaration to the persons involved in the research may not be restricted or excluded by any ethical, legal or regulatory provisions, national or international.

9. Medical research should be conducted in such a way that it minimizes potential harm to the environment.
10. Medical research involving human beings should be conducted only by persons with appropriate education, training, and qualifications in ethics and science. Research involving patients or healthy volunteers require the supervision of a physician or other qualified and competent health professional.
11. Adequate opportunities to participate in medical research should be available to under- represented groups.
12. Physicians who associate medical research with medical care should involve their patients in research only to the extent that it is justified by its potential value in prevention, diagnosis or treatment and if physicians have good reason to think that participation in research will not affect the health of the patients concerned.
13. Adequate compensation and treatment must be guaranteed for those who have suffered harm as a result of their participation in a search.

Risks, constraints, and benefits

1. In medical practice and medical research, most interventions involve risks and inconveniences.
2. Medical research involving humans can only be conducted if the importance of the objective exceeds the risks and inconveniences for those involved.
3. Any medical research involving humans must first be carefully evaluated for foreseeable risks and harms to the persons and groups involved, in relation to the foreseeable benefits to them and other persons or groups affected by the disease. pathology studied.
4. All measures to reduce the risks must be implemented. The risks must be constantly monitored, evaluated and documented by the researcher.
5. Physicians cannot engage in research involving humans without the certainty that the risks have been properly assessed and can be managed satisfactorily.
6. When the risks outweigh the potential benefits or when definitive conclusions have been established, physicians must assess whether they continue, modify or immediately stop a search.
7. Vulnerable people and people.
8. Some groups or individuals being researched are particularly vulnerable and may have a higher likelihood of being abused or suffering additional harm.
9. All groups and vulnerable people should be given adequate protection.
10. Medical research involving a vulnerable group is justified only if it meets the health needs or priorities of that group and cannot be carried out in a non-vulnerable group. In addition, this group should benefit from the knowledge, practices or interventions that result.

Scientific requirements and research protocols

1. Medical research involving humans must comply with generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information and appropriate laboratory experiments and, where appropriate, about animals. The welfare of animals used in research must be respected.
2. The design and conduct of all research involving humans should be clearly described and justified in a research protocol.
3. This protocol should contain a statement on the ethical issues in writing and should indicate how the principles of this Declaration have been taken into consideration. The protocol should include information about

funding, promoters, institutional affiliations, potential conflicts of interest, incentives for those involved in research, and information about the measures planned to treat and/or compensate those who have suffered prejudice because of their participation in the research.
4. In clinical trials, the protocol should also mention the appropriate arrangements for access to the intervention tested after the clinical trial.

Research Ethics Committees

1. The research protocol must be submitted to the relevant research ethics committee for evaluation, comment, advice, and approval before the research begins. This committee must be transparent in its operation, independent of the researcher, the promoter and any other undue influence and must be properly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is conducted, as well as international norms and standards, but these must not be allowed to restrict or exclude any of the protections guaranteed by this Declaration people involved in the research.
2. The committee must have a right to follow-up on ongoing research. The researcher must provide the committee with information on follow-up, including any serious adverse events. No changes can be made to the protocol without evaluation and approval by the committee. At the end of the study, the researchers must submit a final report containing the summary of the findings and conclusions.

Privacy and confidentiality

Every precaution must be taken to protect the privacy and confidentiality of personal information about those involved in the research.

Informed consent

1. The participation of persons capable of giving informed consent to medical research must be a voluntary act. While it may be appropriate to consult with family members or community leaders, no one capable of giving informed consent can be involved in research without giving free and informed consent.
2. In medical research involving individuals who are capable of giving informed consent, any person who may potentially be involved must be properly informed of the objectives, methods, sources of funding, any potential conflict of interest, institutional affiliation of the researcher, the expected benefits and potential risks of the research, the inconvenience it may cause, the measures that will be taken after the clinical trial and any other relevant aspect of the research. The person potentially involved in the search must be informed of his / her right to refuse to participate or withdraw at any time without retaliation. Particular attention should be given to the specific information needs of each person potentially involved in the research as well as the methods adopted to provide the information. When the physician or other qualified person is satisfied that the data subject has understood the information, he or she should seek free and informed consent, preferably in writing. If consent cannot be given in writing, unwritten consent must be formally documented in the presence of a witness.
3. All persons involved in medical research should have the choice to be informed of the general conclusions and results of these.
4. When seeking the informed consent of a person to participate in a search, the physician must be particularly attentive when the latter is in a relationship of dependency with him or may consent under duress. In this case, informed consent must be sought by a qualified person who is completely independent of this relationship.
5. Where the research involves a person who is incapable of giving informed consent, the physician must seek the informed consent of his or her legal representative. People who are incapable should not be included in research

that has no chance of benefiting them unless it aims to improve the health of the group they represent, that it cannot be achieved with capable people to give informed consent and that there are only minimal risks and disadvantages.

6. Where a person considered incapable of giving informed consent is able to consent to his participation in the research, the physician shall seek such consent in addition to the consent of his legal representative. The refusal of the person potentially involved in the research should be respected.
7. Research involving persons who are physically or mentally incapable of giving consent, such as unconscious patients, may be conducted only if the physical or mental condition preventing informed consent is a necessary characteristic of the group being researched. In such circumstances, the physician must seek the informed consent of the legal representative. In the absence of a legal representative and if the research cannot be delayed, it can be started without informed consent. In this case, the research protocol must mention the specific reasons for involving people whose condition renders them incapable of giving informed consent and the research must be approved by the research ethics committee concerned. The consent to keep the person concerned in the search must, as soon as possible, be obtained from the person himself or his legal representative.
8. The physician must provide complete information to the patient about the nature of the care related to the research. The refusal of a patient to participate in a research or his decision to withdraw from it must never interfere with the patient-doctor relationship.
9. For medical research using tissues or data of human origin, such as tissue and data research in biobanks or similar repositories, physicians must seek informed consent for their analysis, storage and/or reuse. Exceptional situations may arise where it is impractical or impossible to obtain consent. In such situations, research may be undertaken only after evaluation and approval by the relevant research ethics board.

Use of placebo

The benefits, risks, harms, and effectiveness of a new intervention must be tested and compared to those of proven best interventions except under the following circumstances:

a) where there is no proven intervention, the use of placebo, or non-intervention, is acceptable; or
b) when for essential methodological and scientifically based reasons the use of any intervention less effective than the best tested, the use of a placebo, or the non-intervention, is necessary in order to determine the effectiveness or safety of an intervention,
   and when patients receiving a less effective intervention than the best-tested, placebo, or non-intervention, they are not at risk of further serious or irreversible harm by not having received the best intervention.
c) Great care must be taken to avoid any abuse of this option. Conditions of access to the intervention tested after the clinical trial.
d) In anticipation of a clinical trial, sponsors, researchers, and host governments should make provisions for all participants who still need an intervention identified as beneficial in the trial to access it after this one. This information must also be communicated to participants during the informed consent process.

Registration of research, publication, and dissemination of results

Any research involving humans must be recorded in a publicly accessible database before the first person involved in the research is recruited.

Researchers, authors, promoters, editors, and publishers all have ethical obligations regarding the publication and dissemination of research results. Researchers have a duty to make available to the public the results of their research involving humans. All parties are responsible for providing complete and accurate reports. They should adhere to accepted ethical guidelines for report writing. Results that are both negative and inconclusive and positive should be published or made public by other means. The publication should mention sources of funding, institutional affiliations, and conflicts of interest. Research reports that do not conform to the principles of this Declaration should not be accepted for publication.

Unproven interventions in clinical practice

In the context of the treatment of a patient, for lack of proven interventions or lack of effectiveness of these interventions, the doctor, after having sought the advice of experts and with the informed consent of the patient or his legal representative, may resort to an unrecognized intervention if, in his or her professional judgment, it offers a chance to save lives, restore health or alleviate the suffering of the patient. This intervention should then be researched to assess its safety and effectiveness. In any case, the new information must be recorded and, where appropriate, made public.

The typographies of paragraphs 26 and 32 were corrected by the WMA Secretariat on 27 June 2014. Other typographies in the statement were corrected by the Secretariat on 4 December 2014.

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