BANDO AIFA RI

FINAL STUDY REPORT (FSR)
Structure and Content of Clinical Study Reports. ICH E3. (Modified)

1.   Title Page
1.1   **Title**

Title: Group sequential response adaptive randomized clinical trial of chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer

Short title/Acronym: CDK4/6-inhibitor or chemotherapy, in combination with ENDOcrine therapy, for advanced breast cancer / KENDO

Test drug: Chemotherapy regimens were at the discretion of the treating physician, based also on patient's features and preferences. Possible chemotherapy regimens are: anthracycline plus taxane, taxane-based (without anthracycline), anthracycline-based (without taxane), capecitabine- (or other fluoropyrimidines)-based, and others.
Endocrine therapy: non-steroidal Aromatase Inhibitor (anastrozole, letrozole) or steroidal Aromatase Inhibitor (exemestane) or fulvestrant. CDK4/6 inhibitors (palbociclib, ribociclib or abemaciclib).

Non pharmacological treatment: none

Study design.

Disease: **Treatment**

Luminal (HER2-negative) locally advanced or **metastatic breast cancer (ABC)**, with features of aggressiveness

Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy

Permuted Block Randomization (until two relapses are experienced in both arms)

Response Adaptive Randomization (the allocation probabilities updating process will take place after 105 and 128 patients are enrolled along with interim analysis on efficacy allowing for early stopping of the trial)

Treatments:
CT:
● anthracyclines + taxanes
● taxanes
● anthracyclines
● fluoropyrimidines
● others
ET:
● non-steroidal AI
● steroidal AI
● fulvestrant
CKK4/6 inhibitor:
● palbociclib
● ribociclib
● abemaciclib

Arm B: concomitant chemotherapy plus endocrine therapy

Treatments will continue until disease progression or toxicity or patient refusal

Cross-over is encouraged (although not mandatory)

This is a prospective, open label, multicenter, phase 2, group sequential response adaptive randomized trial, comparing two combination treatments for locally advanced or metastatic HRpositive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness:
- **Arm A:** concomitant CDK4/6 inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant).
- **Arm B**: chemotherapy plus endocrine therapy (AI or fulvestrant). The chemotherapy regimen was at the discretion of the treating physician and was administered for at least 4-6 months (unless there is toxicity or disease progression). The endocrine agent can be started concomitantly with chemotherapy or sequentially, after stopping chemotherapy.
Treatments continued until disease progression or toxicity or patient refusal.
Cross-over to the other treatment arm was suggested (but not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy within the study, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy within the study.
The primary aim is to compare the efficacy of concomitant CDK4/6 inhibitor plus endocrine therapy versus chemotherapy plus endocrine therapy (administered either concomitantly from the beginning or sequentially) in terms of progression-free survival (PFS).

Study Code: FARM125RHR
Protocol Code: IRST174.19 (L2P1388)
Eudract Number: 2016-004107-31
Phase: II
Date of Contract: 07/02/2017
Date of Ethic Committee approval:
Ethical Approval by Coordinating Centre's Ethics Committee (CEC): 12/07/2017
Approval of Amendment 1.0 dated 09/11/2018 by CEC : 12/12/2018

Period covered:
Date of First patient entered: 26/07/2017
Date of last patient completed: 29/12/2021

Date of early termination: 31/12/2021

Name of report authors: Emanuela Scarpi, Bernadette Vertogen, Anna Miserocchi, Ottavia Tartagni

Name, status and affiliation of principal/co-ordinating, investigator: Ugo De Giorgi, M.D.. Head of Breast Cancer Unit, Department of Medical Oncology, IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" – IRST S.r.l. Via P. Maroncelli 40, 47014 Meldola (FC) – Italy

The responsible Investigator ensured that this study was conducted in compliance with the protocol, following the instructions and procedures described in it, adhering to the principles of Good Clinical Practice and to current local legislation, and in accordance with:
- the principles laid down by the 18th World Medical Assembly (Helsinki, last amended October 2000, with additional footnotes added 2002 and 2004)
- ICH Harmonized Tripartite Guidelines for Good Clinical Practice (CPMP/ICH/135/95) 1996
- Directive 2001/20/EEC of the European Parliament and other relevant local legislation and applicable regulatory requirements Subject data protection.

1.2   Study Administration and Investigators

Name and affiliation of Principal Investigator: Dr. Ugo De Giorgi, IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" – IRST S.r.l. Via P. Maroncelli 40, 47014 Meldola (FC) – Italy

Name, affiliation and role in the study of all other investigators:

See appendix 1 for completed list of participating trial centres including sites not activated and names and affiliations of all investigators involved in the study.

CV of all Investigators are available for review on request from the study sponsor.

Name and affiliation of laboratories used in the study:
●   **Laboratorio di Bioscienze IRCCS** Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" – IRST S.r.l. Via P. Maroncelli 40, 47014 Meldola (FC) – Italy

●   **Laboratorio DME-CTC IOV** - Istituto di Ricerca Pediatrica Città della Speranza, Corso Stati Uniti 4 - 35129 Padova

Name and affiliation of contract pharmaceutical/research organization: Not Applicable.

Name and address of relevant Sponsor study personnel: Unit of Biostatistics and Clinical Trials, IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" - IRST S.r.l. , Via P. Maroncelli 40, 47014, Meldola, Italy

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1.4 List of Abbreviations and Definition of Terms

ABC Advanced Breast Cancer
AE Adverse Event
AI Aromatase Inhibitor
ALT Alanine aminotransferase (previously SGPT)
ANC Absolute neutrophil count ASAT
Aspartate aminotransferase (previously SGOT)
AR Adverse reaction
BSA Body surface area
CBC Complete blood count
CC Coordinating Centre
CEC Coordinating Center's Ethics Commitee
CI Confidence interval
CNS Central Nervous System
CT Chemotherapy
CI Chief Investigator
CRA Clinical Research Associate (Monitor)
CRO Contract Research Organisation
CTscan Computed tomography
CTC Common toxicity criteria
CTCs Circulating Tumor Cells
DFS Disease Free Survival
DFI Disease Free Interval
ECG Electrocardiogram
ECOG-PS Eastern Cooperative Oncology Group Performance status
eCRF electronic Case Report Form
ET Endocrine Therapy
FPFV First Patient First Visit
GCP Good Clinical Practice
G-CSF Granulocyte colony stimulating factor
GP General Practitioner
IB Investigator's Brochure
ICF Informed Consent Form
ICH International Conference of Harmonisation
IDMC Independent Data Monitoring Committee
IEC Independent Ethics Committee
IMP Investigational Medicinal Products
IRB Independent Review Board
LPFV Last Patient First Visit
LP Last Patient
LPLV Last Patient Last Visit
MRI Magnetic Resonance Imaging
ORR Overall Response Rate
OS Overall survival
PD Progressive disease
PFS Progression Free Survival
PI Principal Investigator
PIL Participant/ Patient Information Leaflet
PR Partial response

QoL Quality of Life questionnaires
RECIST Response Evaluation Criteria In Solid Tumors
SAE Serious Adverse Event
SAR Serious Adverse Reaction
SD Stable disease
SOP Standard Operating Procedure
SSN Sistema Sanitario Nazionale
SUSAR Suspected Unexpected Serious Adverse Reactions
TPFS Total Progression Free Survival
TTP Time to progression
ULN Upper limit of normal
WBC White blood cells
WHO World Health Organization

2 Synopsis

Title	Group sequential response adaptive randomized clinical trial of concomitant chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer - IRST174.19 KENDO	
Phase	Phase II	
Study Centers	20 centers	
Principal Investigator (Coordinating Investigator)	Ugo De Giorgi	
Study Period	First subject enrolled(FPFV): 26/07/2017 Last patient completed (LPFV): 29/12/2021	
Objectives	Primary	To compare the efficacy of concomitant CDK4/6 inhibitor plus endocrine therapy versus chemotherapy plus endocrine therapy (administered either concomitantly from the beginning or sequentially) in terms of progression-free survival (PFS).
	Secondary	To compare between treatment arms: • quality of life (EORTC QLQ-C30 and QLQ-BR23) • toxicity (CTCAE version 5.0)

		• time to treatment failure • best response rate • duration of response • clinical benefit rate • overall survival (OS) • PFS and clinical benefit with the subsequent line of treatment after cross-over: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy • correlative biomarkers of response to CDK4/6 inhibitors and chemotherapy: – tissue markers (on the primary tumor and / or metastatic tissue) – circulating markers (e.g. CTCs, ctDNA) To compare, within the experimental arm, PFS with concomitant versus sequential administration of chemotherapy and endocrine therapy (exploratory endpoint).
Methodology		Study Design: Prospective, open label, multicenter, group sequential response adaptive randomized phase II study, comparing two treatments for locally advanced or metastatic luminal breast cancer: - Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (aromatase inhibitor [AI] or fulvestrant) - Arm B: chemotherapy plus endocrine therapy (AI or fulvestrant, administered either concomitantly from the beginning of chemotherapy or sequentially after 4-6 months of chemotherapy) Treatments will continue until disease progression or toxicity or patient refusal. Cross-over to the other treatment arm is encouraged (although not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy. Study population: postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not) with HR-positive, HER2-negative, locally advanced or metastatic breast cancer, with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.

Inclusion criteria:

· Histological diagnosis of HR-positive (ER ≥10% of tumor cells), HER2-negative (according to ASCO guidelines 2018) breast cancer, determined by local laboratory on most recent available tumor tissue.
· Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as "advanced breast cancer (ABC)").
· At least one of the following signs of disease aggressiveness:
o The main criteria are a low expression of ER ($10\% \leq ER < 50\%$) and/or a relapse while on the first 2 years of adjuvant endocrine therapy or disease progression (PD) within the first 6 months of first-line endocrine therapy for ABC
o Other tumor characteristics of aggressiveness that make the patient potentially candidate to chemotherapy, according to the guidelines of the Italian Association of Medical Oncology [AIOM guidelines 2017], such as: elevated Ki67 (preferably documented, if available, on a metastatic biopsy), low expression of hormone receptors (e.g. progesterone receptor <20%), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms; these patients are eligible if chemotherapy is considered a suitable option by the treating physician.
· Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not).
· Measurable disease according to RECIST 1.1 criteria, or not measurable but evaluable disease.
· Any prior adjuvant chemotherapy or endocrine therapy
· No prior chemotherapy for advanced disease.
· Up to one prior line of endocrine therapy for ABC.
· Age ≥ 18 years.
· Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).
· Adequate organ (renal, hepatic, bone marrow, cardiac) functions.
· Female participants of child bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.
· Participant is willing and able to give informed consent for participation in the study.

Exclusion criteria:

· Any prior chemotherapy or CDK4/6 inhibitor for advanced breast cancer
· More than 1 prior line of endocrine therapy for ABC.

	· Patients who have not recovered from adverse events due to prior therapies to grade ≤1 (excluding alopecia). · Active central nervous system metastases. · History of allergic reactions attributed to compounds of similar chemical or biologic composition to the drugs used in the study. · Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. · Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years. Visits and assessments: ● Visit at screening: physical examination, laboratory test and tumor evaluation for eligibility; QoL questionnaires ● During treatment, before every cycle physical examination, laboratory test, AE and concomitant medication assessment, study drug compliance ● During treatment, every three months: tumor evaluation and biomarker analysis; QoL ● End of treatment: physical examination, laboratory test, AE and concomitant medication assessment, study drug compliance, tumor evaluation, QoL Follow up: as per clinical practice
Number of Subjects	Actual sample size depended on the possibility of early stopping for efficacy at any of the two planned interim analyses. However, at the most an overall sample size of 150 patients was planned. Patients were allocated according to group sequential response adaptive randomization. 49 patients were analysed.
Study Product, Dose, Route, Regimen and duration of administration	Chemotherapy regimen: at the discretion of the treating physician (treatment of physician choice, TPC), based also on patient's features and preferences. Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard". Chemotherapy regimens will be classified as: ● anthracycline + taxane, ● taxane, ● anthracycline, ● capecitabine / fluoropyrimidines, ● others.

	Endocrine therapy:
	• non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI
	• non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed while on adjuvant steroidal AI
	• steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed while on adjuvant non-steroidal AI
	• fulvestrant in women not pretreated with fulvestrant for advanced disease
	CDK4/6 inhibitor:
	• palbociclib
	• ribociclib
	• abemaciclib
	Any cytotoxic or endocrine agent to which a patient was exposed in the adjuvant setting may be administered within this study only if PD occurred at least 12 months after the end of that drug.
Reference therapy	The standard reference therapy is CDK4/6 inhibitor plus endocrine therapy.
Statistical Methodology	The patients will be allocated according to block randomization until two events are observed in each arm, and then according to the time-to-event adaptation (Zhang and Rosenberger, 2007) of the group sequential Doubly-adaptive Biased Coin Design (DBCD) whose allocation probabilities are computed at the end of the block randomization and after around 70% and 85% of the 150 maximum patients enrolled during a 23 month period. At these last two (i.e. after 105 and 128 patients, respectively), interim analysis on efficacy (Zhu and Hu, 2010) will be carried out allowing for early stopping. Assuming for the survival times an exponential distribution parametrized in terms of its expected value, the null hypothesis of equality of PFS versus a higher one for arm B () will be tested by means of the nonparametric log-rank test with a 10% significance level. The adoption of the Lan and DeMets (1983) - spending function for determining the upper boundaries allows to preserve the nominal level throughout the two interim analyses. At the end of the 16-month follow up, administrative censoring is introduced. Therefore, the initial total study duration is 39 months.
	Simulations carried out assuming different scenarios showed good operating characteristics, especially when compared with the ones of the usual complete randomization (CR) coupled with the same test statistics on a fixed 150 patient sample size. Previous results on palbociclib and fulvestrant combination in second line (Paloma 3

Study, supplementary material of Turner NC, NEJM 2018) and the characteristics of our target population lead us to assume a median PFS of 8 and 12 months for arm A and B, respectively. Under this scenario, for a sample size of at the most 150 patients, the proposed design strategy has led to a simulated power of 0.911 compared with a 0.717 one for the CR design. Moreover, a reduced expected sample size (ESS) of 121.259 patients is observed due to early stopping for efficacy, whereas CR forces all 150 patients to be equally assigned to both arms. Furthermore, assuming a median PFS of 8 and 12 months for Arm A and B, respectively, simulations showed that group sequential DBCD allocates around 56% of the patients to Arm B.

The Intention-to-treat (ITT) population is defined as the population of all enrolled patients. The activity (AP) and the safety population (SP) are considered as all patients who received, in each treatment group, at least one dose of treatment. Descriptive statistics will be reported for patients and tumors characteristics. Proportions will be compared with chi-square or Fisher exact test as indicated, and continuous variables will be compared by t-test or non parametric tests according to data distributions, providing 95% confidence intervals (95% CI). Time to event data will be analyzed using Kaplan-Meier curves, with 95% CI for median time and for each year of follow-up calculated with nonparametric methods. Comparisons between the two treatment arms will be performed using the log rank test, at a significance level of 10%. Unadjusted and adjusted hazard ratios (HR) will be calculated using Cox proportional-hazard models, including or not the main know prognostic and predictive factors. Two-sided 95% CI for each HR will be provided.

No stratification factor will be considered in the primary endpoint analysis.
The maximally selected rank statistics (MSRS) method was used to identify exploratory cut-offs to determine for each genomic signature/gene of interest high and low levels of expression, so to assess their association with PFS or OS. Missing data were assumed to be missing completely at random for all analyses and no imputation was done to estimate missing observations. Since the study did not reach the required sample size, all the analyses presented in this report are intended to be hypothesis-generating, with a significance level set at $p<0.05$, without adjustments for multiplicity.
Baseline gene expression difference between the two treatment cohorts was assessed with two-class unpaired SAM analysis, with a false discovery rate (FDR)≤5%.

All statistical analyses were performed with R vers.3.6.1 and SPSS vers.24 (IBM Corp. Released 2016. IBM SPSS Statistics for MacOS, Version 24.0. Armonk, NY: IBM Corp.) for MacOSX.

Efficacy	Among 49 randomized patients (median follow-up: 35.2 months), median progression-free survival (mPFS) with chemotherapy+ET (11.2 months, 95% confidence interval[CI]: 7.7-15.4) was numerically shorter than mPFS (19.9 months, 95%CI: 9.0-30.6) with CDK4/6i+ET (hazard ratio: 1.41, 95%CI: 0.75-2.64). PAM50 Basal-like tumors under CDK4/6i+ET exhibited worse PFS (mPFS: 11.4 months, 95%CI: 3.00-not reached [NR]) and overall survival (OS) (mOS: 18.8 months, 95%CI: 18.8-NR) compared to other subtypes (mPFS: 20.7 months, 95%CI: 9.00-33.4; mOS: NR, 95%CI: 24.4-NR). In the chemotherapy arm, Luminal A tumors showed poorer PFS (mPFS: 5.1 months, 95%CI: 2.7-NR) than other IS (mPFS: 13.2 months, 95%CI: 10.6-28.1). Genes/pathways involved in BC cell survival and proliferation were associated with worse outcomes, compared to genes involved in immunological signatures, especially in the CDK4/6i arm. *CD24* was the only gene significantly associated to worse PFS in both arms. Tertiary lymphoid structures and higher tumor-infiltrating lymphocytes also showed favorable survival trends in the CDK4/6i arm.
Safety	No patient stopped the treatment due to toxicity, withdrawal or other causes unrelated to disease progression or death. No standard clinicopathological variables were found to be significantly associated with PFS and OS. The most common (≥5%) grade 3–4 AEs in both arms was neutropenia (41.2% in arm A and 22% in arm B, respectively). No patient stopped study treatment because of toxicity. Among patients treated in arm A, 2 of them (12%) required a dose reduction, instead in the arm B, 10 (31%) patients. No serious adverse events have been reported

Overall, the safety profile was consistent with previous literature and manageable, without novel unexpected safety concerns. CT with concomitant ET led to a slight increase in AEs due to the addition of ET-related toxicities. |
| Conclusion | The KENDO randomized phase II trial was designed to demonstrate the superiority of chemotherapy (CT), +/- endocrine therapy (ET), with respect to CDK4/6-inhibitors+ET in patients with HR+/HER2-negative metastatic breast cancer (MBC) exhibiting aggressive characteristics or endocrine resistance. While the trial closed prematurely and failed to meet its primary endpoint, intriguing trends emerged from tissue biomarker analyses. While PAM50 Luminal A tumors showed poor outcomes when treated with CT, Basal-like tumors responded poorly to CDK4/6-inhibitors+ET. Moreover, research-based PAM50 ROR-P categories predicted response to CDK4/6-inhibitors-based regimens. Interestingly, high levels of genes/ signatures of pathways involved in tumor survival and proliferation were associated with worse outcomes, compared to immune-related genes/signatures, especially in the CDK4/6-inhibitors arm. Notably, tertiary lymphoid structures and higher tumor-infiltrating lymphocytes also showed favorable survival trends with CDK4/6-inhibitors+ET. These findings highlight the importance of considering tumor biology and immune composition for optimizing |

	therapeutic strategies in HR+/HER2-negative MBC. Confirmatory studies are required. Biomarker analyses strengthen the role of PAM50 IS for guiding therapeutic choices in this context, highlighting the need to incorporate them in clinical trials for patient stratification or selection; ROR-P categories might be valuable for the same purpose but need further evaluation. Tumor microenvironment immune composition (e.g. TILs, TLS, genomic signatures) might be useful to predict prognosis and therapeutic benefit from CDK4/6-inhibitors+ET but need confirmation in larger studies. *CD19* and/or *CXCL13* mRNA levels might be a useful surrogate of TLS when experienced pathologists are not available. *CD24* was found to be significantly associated with prognosis in both cohorts, warranting further investigation.
Publication reference	Palleschi M. et al. CDK4/6-inhibitors vs. chemotherapy in advanced HR+/HER2-negative breast cancer: results and correlative biomarker analyses of the KENDO randomized phase II trial. *npj Breast Cancer*, 2023. <u>Currently under review.</u>
Date of report	06/10/2023

3 Ethics
3.1 Independent Ethics Committee (IEC)

The Investigator submitted this protocol to the local Ethics Committee.
The EC approval report contains details of the trial (title, protocol number and version), documents evaluated (protocol, informed consent material, advertisement when applicable) and the date of the approval.

For the list of the IECs who approved protocol and relatives amendments, please see appendix 2.

3.2 Ethical Conduct of the Study

The responsible Investigator ensured that this study was conducted in compliance with the protocol, following the instructions and procedures described in it, adhering to the principles of Good Clinical Practice and to current local legislation, and in accordance with:
- the principles laid down by the 18th World Medical Assembly (Helsinki, last amended October 2000, with additional footnotes added 2002 and 2004)
- ICH Harmonized Tripartite Guidelines for Good Clinical Practice (CPMP/ICH/135/95) 1996
- Directive 2001/20/EEC of the European Parliament and other relevant local legislation and applicable regulatory requirements Subject data protection.

3.3 Subject / Patient Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from each subject prior to entering the trial or, where relevant, prior to evaluating the subject's suitability for the study.

The informed consent document used by the Investigator for obtaining the subject's informed consent was approved by the local EthicsCommittee.

The Investigator explains the study procedures to the patients and leaves him/her a copy of the information sheet. The patient is given enough time to discuss the possibility of entering the trial with family members or with the General Practitioner. At the following visit, the Investigator discusses again the study with the patient and, if the patient agrees to participate, he or she dates and signs the informed consent form.

A copy of the patient's signed written consent form is be kept by the center in the proper section of the Investigator Site File.

See appendix 3 for:
- Patient information sheet and Consent Form for the initial protocol (final version 03/03/17)
- Patient information sheet and Consent Form amended, (amendment 1.0 09/11/18)

4 Investigational Plan

The protocol was amended following changes in clinical practice with the advent of new therapeutic options for hormone receptor-positive, HER2-negative metastatic breast cancer (also called "luminal" breast cancer).

The original study included a randomized comparison between concomitant chemo-endocrine therapy and chemotherapy followed by endocrine therapy as first-line treatment of luminal metastatic breast cancer with aggressive features, such as low expression of progestin receptors and/or high proliferative index (luminal B subtype).

When the project was presented there were no molecular targeted drugs registered for this breast cancer subtype and the therapeutic options were endocrine therapy and chemotherapy. It was therefore important to understand whether the concomitant administration of these two treatments (concomitant chemo-endocrine therapy) was more effective than their sequential administration (commonly used in the clinic and considered the "standard" arm of the study).

Recently, a new class of drugs, the cyclin-dependent kinase inhibitors 4 and 6 (CDK4/6 inhibitors) has become available in clinical practice. Given in combination with hormonal drugs, CDK4/6 inhibitors have led to a marked improvement in progression-free survival of patients with metastatic luminal tumors compared to what can be achieved with hormone therapy alone, both in the first treatment line (in combination with an aromatase inhibitor) and in the second line (in combination with fulvestrant). The combination of CDK4/6 inhibitors and hormone therapy also produce objective response rates comparable to those obtained with chemotherapy. These combinations have become standard treatment options in the first and/or second line of hormone therapy.

Therefore, today we cannot ignore the existence of a new "standard" therapy option in metastatic luminal carcinoma: combination therapy with a CDK4/6 inhibitor + an endocrine agent.

It was thus necessary to amend this study, proposing what is currently the most relevant comparison: that between CDK4 / 6 inhibitor + endocrine agent on one side (arm A) and chemotherapy + endocrine therapy on the other (arm B).

The study maintains the pragmatic character it originally had, leaving the clinician the choice of the CDK4/6 inhibitor drug (to be used according to the indications registered at the time of treatment), of the chemotherapy regimen (to be chosen among those considered standard for this breast cancer subtype and treatment line) and of the hormonal agent. The latter will be administered in arm A from the beginning of treatment with CDK4/6 inhibitor, while in arm B it will be at the discretion of the clinician to administer it concomitantly (from the beginning of chemotherapy: concomitant chemo-endocrine therapy) or sequentially (as maintenance therapy, after stopping chemotherapy at the end of 4-6 months of chemotherapy treatment: sequential chemo-endocrine therapy).

It was also decided to use the most efficient study design with group sequential adaptive randomization, moving from a phase 3 to a randomized phase 2 study but conserving adequate power for a comparison of treatment efficacy and introducing the possibility of early termination of the study for efficacy, reducing the expected total number of patients and including more patients in the more effective treatment arm.

The biological part of the study was also appropriately modified to identify specific predictors of response to CDK4/6 inhibitors + endocrine therapy and those to chemo-endocrine therapy.

The promoter has considered this amendment as substantial because it introduces trial design changes, with a significant impact on patient management.

4.1   Introduction

Although metastatic luminal breast cancer is most often treated with endocrine agents as first-line therapy, chemotherapy may be useful in specific conditions, and is eventually administered to most patients after the onset of endocrine resistance. The choice and sequence of treatments for metastatic luminal breast cancer depends on responsiveness to previous (e.g. adjuvant) therapies and on biological and clinical features. Chemotherapy is used in earlier lines of treatment in presence of signs of disease aggressiveness, such as short disease-free interval, elevated Ki67 (preferably, if available, on a metastatic biopsy), low expression of hormone receptors (HRs), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms. Several new targeted agents are being developed in breast cancer, and some of them have been shown to improve the efficacy of endocrine therapy when given in combination with endocrine agents, and to overcome or delay the development of endocrine-resistance. The cyclin-dependent Kinase 4 and 6 (CDK4/6) inhibitors (palbociclib, ribociclib, abemaciclib) significantly improve progression-free survival when given in combination with an aromatase inhibitor (AI) as first-line treatment of endocrine-sensitive metastatic breast cancer, or when given in combination with fulvestrant in patients with HR-positive metastatic breast cancer after progression to an aromatase inhibitor. Therefore, a combination of an endocrine agent (AI or fulvestrant) and a CDK4/6 inhibitor has become the preferred first-line treatment in patients with HR-positive metastatic breast cancer, except in cases of very indolent disease or poor performance status, when endocrine therapy alone is still preferred, or in cases of very aggressive disease which may still be treated with first-line chemotherapy. The combinations of CDK4/6 inhibitors and endocrine therapy could potentially replace chemotherapy alone in some cases of aggressive disease, and studies comparing the two strategies are ongoing. Preclinical studies show a synergism between chemotherapy and some endocrine agents such as AIs and fulvestrant. Therefore, a combination of chemotherapy and endocrine therapy could be a further, potentially very active, treatment strategy in metastatic luminal breast cancer. The choice between a chemotherapy-based and a CDK4/6 inhibitorbased treatment remains particularly controversial in patients with doubtful endocrine sensitivity, e.g. due to a low expression of estrogen receptors (ERs), or of primary endocrine resistance, indicated by a short disease-free interval. We plan to conduct a phase II group sequential response adaptive randomized clinical trial comparing the combination of chemotherapy plus endocrine therapy with CDK4/6 inhibitors plus endocrine therapy in patients with advanced HR-positive, HER2-negative breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option. The main aim is to assess if a combination of

chemotherapy and endocrine therapy is superior to CDK4/6 inhibitors plus endocrine therapy in terms of progression-free survival. Secondary aims are comparing the two treatment arms in terms of disease control rate (objective response or stable disease after 3 months of treatment), objective response rate, overall survival, toxicity and patient reported outcomes. In a biological correlative study, the expression and mutational/ Copy Number Variation (CNV) profiles of genes involved in pathways subtending cell cycle progression (cyclin D – CDK4/6 – Rb), as targets of CDK4/6 inhibitors, and response to chemotherapy (p53, p38, DNA damage response) will be assessed on tumor specimens. The same gene mutational profiles and CNVs will be assessed on Circulating Tumor Cells (CTC) with analysis at single cell level. Also, on a subgroup of patients enrolled at Meldola, Forlì and Cesena, the expression profiles of genes involved in the pathways of interest (cyclin D – CDK4/6 – Rb, p53, p38, DNA damage response) will be assessed on CTCs at single cell level.

4.2 Study Objectives

Primary objective
To compare the efficacy of concomitant CDK4/6 inhibitor plus endocrine therapy versus chemotherapy plus endocrine therapy (administered either concomitantly from the beginning or sequentially) in terms of progression-free survival (PFS).

Secondary objectives
To compare between treatment arms:
• quality of life (EORTC QLQ-C30 and QLQ-BR23)
• toxicity (CTCAE version 5.0)
• time to treatment failure
• best response rate
• duration of response
• clinical benefit rate
• overall survival (OS)
• PFS and clinical benefit with the subsequent line of treatment after cross-over: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy
• correlative biomarkers of response to CDK4/6 inhibitors and chemotherapy:
– tissue markers (on the primary tumor and/or metastatic tissue)
– circulating markers (e.g. CTCs, ctDNA)
To compare, within the experimental arm, PFS with concomitant versus sequential administration of chemotherapy and endocrine therapy (exploratory endpoint).

4.3 Study Design

This is a prospective, open label, multicenter, phase 2, group sequential response adaptive randomized trial, comparing two combination treatments for locally advanced or metastatic HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness:
  - Arm A: concomitant CDK4/6 inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant).
  - Arm B: chemotherapy plus endocrine therapy (AI or fulvestrant). The chemotherapy regimen were at the discretion of the treating physician and were administered for at least 4-6 months

(unless there is toxicity or disease progression). The endocrine agent can be started concomitantly with chemotherapy or sequentially, after stopping chemotherapy.

Treatments were continued until disease progression or toxicity or patient refusal.

Cross-over to the other treatment arm was suggested (but not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy within the study, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy within the study.

Study Drug
Commercial batches for all drugs were used for the purposes of this study.

Subject population and number of subjects planned for inclusion
Patient population: postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not) with HR-positive, HER2-negative, locally advanced or metastatic breast cancer, with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.

Actual sample size will depend on the possibility of early stopping for efficacy at any of the two planned interim analyses. However, at the most an overall sample size of 150 patients is planned. Patients will be allocated according to group sequential response adaptive randomization.

Level and method of blinding
This is an open-label randomized phase II study. As the primary endpoint of our study is the Progression-Free Survival, blinding was considered not necessary.

Control group
Arm B: chemotherapy plus endocrine therapy (AI or fulvestrant).

Study configuration
Parallel groups

Sequence and duration of each study period
Patients were enrolled and randomized after the screening period (which lasted no longer than 28 days), were followed during treatment with visits for evaluation of toxicity at each cycle and with imaging for disease evaluation at least every 3 months until disease progression or until exit from the study for toxicity or patient refusal.
Patients' enrolment was expected to occur over a period of 23 months, with further 16 months of follow up.

Method and timing, of randomisation
All patients, for whom eligibility criteria have been verified, were randomized by the Biostatistics and Clinical Trial Unit of IRST (Coordinating Centre, CC).
Patients were randomized on a 1:1 allocation rate according to block randomization (block of size two) until two events (i.e. disease progression or death) are experienced in both arms and then according to a group sequential DBCD [Hu F 2004] targeting Neyman allocation for time to event data [Zhang L 2007]. The allocation probabilities are computed at the end of the block randomization and updated after 70% and 85% of the maximum 150 patients are enrolled (i.e. 105 and 128 patients). Central separated randomization lists were generated at the start of the trial and then at each halt. The chemotherapy, CDK4/6 inhibitor and endocrine agents were chosen by the treating physician for each patient before randomization.

Treatment administration should begin within 72 hours of the date of randomization. No blinding was planned.

Involvement of any Steering, Committees, together with their function
No Steering Committee was involved.

Interim analyses and justification

The patients were allocated according to block randomization until two events are observed in each arm, and then according to the time-to-event adaptation (Zhang and Rosenberger, 2007) of the group sequential Doubly-adaptive Biased Coin Design (DBCD) whose allocation probabilities are computed at the end of the block randomization and after around 70% and 85% of the 150 maximum patients are enrolled during a 23 month period. At these last two (i.e. after 105 and 128 patients, respectively), interim analysis on efficacy (Zhu and Hu, 2010) will be carried out allowing for early stopping. Assuming for the survival times an exponential distribution parametrized in terms of its expected value, the null hypothesis of equality of PFS versus a higher one for arm B () will be tested by means of the nonparametric log-rank test with a 10% significance level. The adoption of the Lan and DeMets (1983) -spending function for determining the upper boundaries allows to preserve the nominal level throughout the two interim analyses. At the end of the 16-month follow up, administrative censoring is introduced. Therefore, the total study duration is 39 months.

Simulations carried out assuming different scenarios showed good operating characteristics, especially when compared with the ones of the usual complete randomization (CR) coupled with the same test statistics on a fixed 150 patient sample size. Previous results on palbociclib and fulvestrant combination in second line (Paloma 3 Study, supplementary material of Turner NC, NEJM 2018) and the characteristics of our target population lead us to assume a median PFS of 8 and 12 months for arm A and B, respectively. Under this scenario, for a sample size of at the most 150 patients, the proposed design strategy has led to a simulated power of 0.911 compared with a 0.717 one for the CR design. Moreover, a reduced expected sample size (ESS) of 121.259 patients is observed due to early stopping for efficacy, whereas CR forces all 150 patients to be equally assigned to both arms. Furthemore, assuming a median PFS of 8 and 12 months for Arm A and B, respectively, simulations showed that group sequential DBCD allocates around 56% of the patients to Arm B.

Prohibited concomitant medication
Avoid the concomitant use of CDK4/6 inhibitors with strong CYP3A inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, posaconazole, voriconazole, boceprevir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir, conivaptan, telithromycin, nefazodone; grapefruit or grapefruit juice), which lead to increased plasma exposure of CDK4/6 inhibitors. If coadministration of a strong CYP3A inhibitor cannot be avoided, reduce the dose of CDK4/6 inhibitors.
Avoid concomitant use of CDK4/6 inhibitors with strong CYP3A inducers (e.g., phenytoin, rifampin, carbamazepine, enzalutamide, and St John's Wort), which decrease the plasma exposure of CDK4/6 inhibitors.
The use of ribociclib must be avoided in concomitance with drugs with a known potential to prolong QT such as antiarrhythmic medicines (including, but not limited to amiodarone, disopyramide, procainamide, quinidine and sotalol), and other drugs that are known to prolong the QT interval (including, but not limited to, chloroquine, halofantrine, clarithromycin, haloperidol, methadone, moxifloxacin, bepridil, pimozide and ondansetron).

Protocol amendments

The protocol was amended following changes in clinical practice with the advent of new therapeutic options for hormone receptor-positive and HER2 (epidermal growth factor receptor 2)-negative metastatic breast cancer (also called luminal breast cancer).

The original study involved a randomized comparison between concomitant chemo-endocrinotherapy and chemotherapy followed by endocrinotherapy as first-line treatment of luminal metastatic breast cancer with aggressive aspects, such as low expression of the progestin receptor and/or high proliferative index (luminal B subtype).

At the time the project was presented there were no molecularly targeted drugs registered for this subtype of breast cancer and the therapeutic options were hormonal and chemotherapy. It was therefore relevant to understand whether the concomitant administration of these two treatments (chemo-endocrine therapy) was more effective than their sequential administration (commonly used in clinics and considered the "standard" arm of the study).

At the time of the amendment, a new class of drugs was made available in clinical practice, the inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6 inhibitors) which, administered in combination with hormonal drugs, led to a clear improvement of disease progression-free survival in patients with metastatic luminal tumors compared to that achievable with hormonal therapy alone, both in the first line of treatment (in association with an aromatase inhibitor) and in the second line (in association with fulvestrant). It was also decided to use the most efficient study design with sequential adaptive randomization in groups, moving from a phase 3 to a randomized phase 2, but maintaining adequate power for a comparison of the effectiveness of the treatments and introducing the possibility of stopping the treatment early. study in case of efficacy, reducing the expected total number of patients and increasing the number of patients enrolled in the most effective treatment arm.

The biological part of the study was also appropriately modified to identify specific predictors of response to CDK4/6 inhibitors + endocrinotherapy and to chemo-endocrinotherapy.

See appendix 4: Protocol Amendment 1.0 09/11/2018 (which includes protocol changes from final version 03/03/2017).
See appendix 5 for the eCRF.

4.4 Selection of Study Population

a) Inclusion Criteria

1. Histological diagnosis of HR-positive (ER ≥10% of tumor cells), HER2-negative (according to ASCO guidelines 2018) breast cancer, determined by local laboratory on most recent available tumor tissue.

2. Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as advanced breast cancer, ABC).

3. At least one of the following signs of disease aggressiveness:

a. the main criteria are a low expression of ER ($10\% \leq ER <50\%$) and/or a relapse while on the first 2 years of adjuvant endocrine therapy or disease progression within the first 6 months of first-line endocrine therapy for ABC b. Other tumor characteristics of aggressiveness that make the patient potentially candidate to chemotherapy, according to the guidelines of the Italian Association of Medical Oncology [AIOM guidelines 2017], such as: elevated Ki67 (preferably documented, if available, on a metastatic biopsy), low expression of hormone receptors (e.g. progesterone receptor <20%) extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms; these patients are eligible if chemotherapy is considered a suitable option by the treating physician.

4. Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not). Postmenopausal status is defined as:

a. bilateral, surgical oophorectomy

b. age ≥60 years

c. age <60 years; with amenorrhea >12 months and follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol concentrations within postmenopausal range

d. age <60 years and previous simple hysterectomy, with FSH, LH and estradiol levels within the post-menopausal range at two consecutive assessments two weeks apart.

5. Measurable disease according to RECIST 1.1 criteria or non-measurable but evaluable lesions.

6. Any prior adjuvant chemotherapy or endocrine therapy

7. No prior chemotherapy for ABC.

8. Up to one prior line of endocrine therapy for ABC.

9. Age ≥18 years.

10. Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2.

11. Adequate organ (renal, hepatic, bone marrow, cardiac) functions.

12. Female participants of child-bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.

13. Participant is willing and able to give informed consent for participation in the study.

b)   Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

1. Any prior chemotherapy or CDK4/6 inhibitor for ABC.

2. More than 1 prior line of endocrine therapy for ABC.

3. Patients who have not recovered from adverse events (AEs) due to prior therapies to grade ≤1 (excluding alopecia).

4. Active central nervous system metastases.

5. History of allergic reactions attributed to compounds of similar chemical or biologic composition to the drugs used in the study.

6. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

7. Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.

c)   Reasons for Withdrawal/Replacement of Study Subjects

Progressive disease (imaging-based), including date of PD
Clinical progressive disease, including date and type of PD

Unacceptable toxicity, including toxicity type
Patient withdrew consent, including reason
Investigator's decision, including reason
Death
Protocol violation, including reason
Lost to follow-up

4.5   Study Materials

All the investigational medicinal products used in the clinical trial are authorised for use in ABC, although the concomitant administration of endocrine agents and chemotherapy is not standard practice.
Any cytotoxic or endocrine agent to which a patient was exposed in the adjuvant setting may be administered within this study only if PD occurred at least 12 months after the end of that drug.
Chemotherapy regimens are at the discretion of the treating physician, based also on the patient's features and preferences.

Possible chemotherapy regimens are:

- based on anthracycline and taxane
- taxane-based (without anthracycline)
- anthracycline-based (without taxane)
- based on capecitabine or other fluoropyrimidines
- other

Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard" per each individual agent's prescribing information. Dosage adjustments during treatment are at the discretion of the treating physician, according to clinical practice.

Endocrine therapy:
- non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI
- non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed while on adjuvant steroidal AI
- steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed on adjuvant non-steroidal AI
- fulvestrant in women not pretreated with fulvestrant for advanced disease

CDK4/6 inhibitor:
- palbociclib
- ribociclib
- abemaciclib

Description of Study Treatment
Possible endocrine treatments are:
- non-steroidal AIs:
○ anastrozole 1 mg daily continuously
○ letrozole 2.5 mg daily continuously
- steroidal AI:
○ exemestane 25 mg daily continuously
- fulvestrant, 500 mg i.m. on days 1, 15, 29 and then every 4 weeks

4.6 Methods for Assigning Subjects to Treatment Groups

All patients for whom eligibility criteria have been verified were randomized by the Biostatistics and Clinical Trial Unit of IRST (Coordinating Centre, CC).

Patients were randomized on a 1:1 allocation rate according to block randomization (block of size two) until two events (i.e. disease progression or death) are experienced in both arms and then according to a group sequential DBCD [Hu F 2004] targeting Neyman allocation for time to event data [Zhang L 2007]. The allocation probabilities were computed at the end of the block randomization and updated after 70% and 85% of the maximum 150 patients are enrolled (i.e. 105 and 128 patients). Central separated randomization lists were generated at the start of the trial and the at each halt. The chemotherapy, CDK4/6 inhibitor and endocrine agents were chosen by the treating physician for each patient before randomization. Treatment administration should begin within 72 hours of the date of randomization. No blinding is planned.

The randomization codes were kept under lock and key in the Biostatistics Unit and were not accessible by anyone else involved in the study with the exceptions of biostatistics.

A copy of the complete randomization lists, giving the randomization number, subject's identification and treatment assigned are attached to the FSR as appendix 6.

Two different randomization lists were produced: the first one, corresponding to the initial version of the protocol (final version 03/03/2017) was stratified per centers; while the second list, after protocol amendment 1.0, was no longer stratified.

All patients were randomized following the standard IRST procedure prior to initiation of study therapy, sending by fax/email the registration form to the Unit of Biostatistics and Clinical Trials. Further detailed information were sent to participating centers and were included in the Investigator Site File.

Each patient was screened according to the study criteria and, if acceptable for entry into the trials, was randomized and a patient code was assigned by study coordinator staff. Patient's code (**center number, subject's number, subject's initials**) was recorded on every page of CRFs.

Patient accrual rates were constantly monitored and action were taken when necessary to resolve recruitment problem.

4.7 Dosage Regimen

Chemotherapy was prescribed as per clinical practice.

Endocrine treatments were prescribed as follows:

· non-steroidal AIs:

o anastrozole 1 mg daily continuously

o letrozole 2.5 mg daily continuously

· steroidal AI:

o exemestane 25 mg daily continuously

· fulvestrant, 500 mg i.m. on days 1, 15, 29 and then every 4 weeks

4.8 Study Blinding

This is an open label study. No blinding was planned.

4.9 Drug Accountability

Commercial batches for all drugs were used for the purposes of this study, according to the indications registered by AIFA.

All treatments were acquired by each participating center pharmacy and reimbursed by SSN.

All participating centers provided separate drug and patient accountability of all study medications. All movements of study medication were documented. The patient was asked to bring all unused medication and used/packaging back to the clinic at each visit, to be returned to the pharmacy.

4.10 Treatment Compliance

Patient's compliance to oral treatment was assessed by the Investigator when the patient returned the drug packaging back to the clinic.

4.11 Prior and Concomitant Medication

Standard premedications for the administration of the cytotoxic chemotherapy was employed in the study. For chemotherapy, recommendations made in the locally approved label were followed for each agent.

Bisphosphonates or a RANK ligand inhibitor could be given according to their product licence and routine clinical practice at the investigator's discretion.

Particular attention must be given to concomitant medications in patients receiving CDK4/6 inhibitors, which have strong potential for drug-drug interactions.

Any medication, other than the study medication taken during the study will be recorded in the eCRF.

Concomitant medications were assessed by the Investigator at baseline, before every cycle and at the end of treatment.

5 Study Assessments (including efficacy and safety variables

A written, signed, informed consent form (ICF) was obtained before any study-specific assessments were initiated.

Screening and Eligibility Assessments

The Screening procedures and assessments were completed within 28 days of Randomization: Data collected during the screening period include:
- Demographics: the date of birth, gender, race.
- Complete medical history: details of any history of prior or concurrent diseases or surgical interventions.
- Confirmation and documentation of HER2 and ER/PgR status on the most recent tumor tissue
- Baseline clinical conditions, including symptoms assessment.
- History of prior treatments and any residual toxicity relating to prior treatment if applicable.

● Concomitant Medication: baseline medications taken within 28 days of Day 1, starting date and prescribing indication.

● Physical Examination, with description (and measurement of the main diameter when feasible) of superficial tumor lesions (or picture if deemed useful).

● ECOG-PS, vital signs (including at least body temperature, resting blood pressure and cardiac frequency), height and weight.

● 12-lead electrocardiogram (ECG), cardiological examination and/or cardiac function assessment by ECHO or MUGA if clinically indicated (recommended for cardiotoxic drugs).

● Laboratory Tests:

o complete blood count (CBC) with differential and platelet count

o blood chemistry assessment including renal and liver function tests (e.g. serum creatinine, potassium, sodium, chloride, calcium, ALAT/SGPT, total bilirubin, alkaline phosphatase, total proteins and albumin)

o urinalysis if clinically indicated.

o serum CA15/3.

o coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR), if clinically indicated.

● Serum or urine pregnancy test within one week prior to the start of study drug for women of child bearing potential.

● Tumor evaluation: CT scan of the chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted. All other clinically indicated examinations (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis).

● Bone scan if clinically indicated (recommended if bone pain is present or bone lesions are found at CT)

● Availability of formalin-fixed, paraffin-embedded tumor specimen (from primary tumor or, when available, from a metastatic biopsy)

● Blood sample for Circulating Tumor Cells (CTCs) analysis

● Blood samples for circulating markers (other than CTCs)

● Quality of life Questionaires: EORTC QLQ-C30 Version 3.0, QLQ-BR23 (breast cancer specific)

Assessments during treatment period (cycle duration 21 or 28 days)

Before each chemotherapy or CDK4/6 inhibitor cycle (within 36 hours prior to treatment administration) the following evaluations were performed:

● Interim medical history, including tumor-related signs and symptoms and new medical conditions

● Assessment of AEs (CTCAE version 5.0);

● Assessment of compliance with study drugs (oral chemotherapy, CDK4/6 inhibitor and/or endocrine therapy)

● Assessment of concomitant medications (in particular if the patient started new medications, registering dosage, route of administration, start and end date and the clinical indication)

● Physical examination.

● ECOG-PS, vital signs (including at least resting blood pressure and cardiac frequency), weight.

● Laboratory Tests: CBC and serum chemistry

<u>Every 3 months</u> (every 4 cycles for 21-day chemotherapy regimens, every 3 cycles for 28-day chemotherapy or CDK4/6 inhibitor regimens) the following evaluations were performed:

● Physical Examination, with description (and measurement of the main diameter when feasible) of superficial tumor lesions
● Tumor assessment with the same radiological exams used to document the disease at baseline:
○ CT scan of the chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly with contrast medium) is permitted.
○ Other exams for tumor assessment if positive at baseline (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis) or as clinically indicated (suspected disease progression at new sites)
● Serum CA15/3
● Quality of life questionnaires:
○ EORTC QLQ-C30 Version 3.0
○ QLQ-BR23 (breast cancer specific)
● Blood samples for circulating markers (other than CTCs)
● Blood samples for CTCs collected 6-8 weeks after the beginning of study treatment, and at the end of treatment.

End of treatment assessments (within 30 days of last treatment administration)

The following assessments were completed within 30 days of the patient's last treatment administration:

● Interim medical history, including tumor-related signs and symptoms and new medical conditions
● Assessment of AEs (CTCAE version 5.0);
● Assessment of compliance with study drugs (oral chemotherapy, CDK4/6 inhibitors and/or endocrine therapy)
● Assessment of concomitant medications (in particular if the patient started new medications, registering dosage, route of administration, start and end date and the clinical indication)
● Physical examination, with description (and measurement of the main diameter when feasible) or picture of superficial tumor lesions.
● ECOG-PS, vital signs (including at least resting blood pressure and cardiac frequency), weight.
● 12-lead electrocardiogram (ECG), cardiological examination and/or cardiac function assessment by ECHO or MUGA if clinically indicated (recommended for cardiotoxic drugs).
● Laboratory Tests: CBC and serum chemistry; urinalysis if clinically indicated.
● Tumor evaluation was required for patients who discontinue study treatment before the first scheduled post-baseline tumor assessment and for patients whose previous tumor assessment did not demonstrate PD and was done more than 2 months prior to the end of treatment visit, with the same radiological exams used to document the disease at baseline:
○ CT scan of chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted.
○ Other exams for tumor assessment if positive at baseline (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis) or as clinically indicated (suspected disease progression at new sites)
● Serum CA15/3

- Quality of life questionnaires for patients whose previous assessment was done more than 2 months prior to end of treatment visit:
  ○ EORTC QLQ-C30 Version 3.0
  ○ QLQ-BR23 (breast cancer specific)
- Blood samples for circulating markers (other than CTCs)

Follow-up visits

After the end of treatment visit, all the patients were followed according to clinical practice. Data on response and TTP to the immediate subsequent line of treatment (particularly in case of cross-over) were registered, along with survival.

For survival data, phone contacts were acceptable.

End of study

The end of study was considered the date of withdrawal of consent, death of the patient or the date of closure of the study, whichever occured first.

Monitoring of Adverse Events

An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered with a pharmaceutical product, which does not necessarily have a causal relationship with this treatment.

An Adverse Reaction (AR) is any untoward and unintended responses to a medicinal product related to any dose.

A Serious Adverse Event (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:
• Death, or
• A life-threatening adverse situation, or
• In-patient hospitalization or prolongation of existing hospitalization, or
• A persistent or significant disability/incapacity, or
• A congenital anomaly/birth defect
Note: the term life-threatening in the definition of "Serious Adverse Event" is defined as any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred.
Medical and scientific judgement should be exercised in deciding whether other important medical events should be considered serious.
Note: 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, or development of drug dependency or drug abuse.

A Suspected Unexpected Serious Adverse Reactions (SUSAR) is a serious adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

Relationship between drug and SAE

The causality relationship between the study drug and the adverse event will be assessed by the investigator as either Yes or No.

If there is any reasonable suspected causal relationship with the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-SAE relationship should be assessed as Yes.

The following criteria should be considered in order to assess YES:

· Reasonable temporal association with drug administration

· Known response pattern to suspected drug

· Disappears or decreases on cessation or reduction in dose

· Reappears on recycle.

The following criteria should be considered in order to assess NO:

· No reasonable temporal association with administration of the drug

· It may have been produced by the patient's clinical state, by environmental or toxic factors, or by other therapies administered to the patient It does not follow a known pattern of response to the suspected drug

· It does not reappear or worsen when the drug is readministered.

Recording and reporting

Non Serious Adverse Events

Non-serious adverse events were recorded on eCRF within 30 days of the last treatment. The investigator should only specify the nature and severity of the event (worst NCI CTCAE v5.0 grade) at each cycle. Any other relevant event not on the checklist was also be recorded on the basis of NCI CTCAE v5.0 toxicity grading.

Serious Adverse Events

The Investigator is responsible for reporting all Serious Adverse Events (SAE), related or not to the study treatment, occurring during the treatment period and within 30 days of the last protocol treatment, to the "Safety Desk". Any late Serious Adverse Drug Reaction (SADR), occurring after this 30-day period, should follow the same reporting procedure.

If a SAE occurs, the following action must be taken by the investigator:

• Fill in the SAE form and send by fax within 24 hours of the initial observation of the event, to the sponsor: IRST Safety Desk, FAX 0543 739288, e-mail: fv.ct@irst.emr.it

• The IRST Coordinating Center (CC) will send the report to national authorities, Ethical Committees and investigators as appropriate, according to local regulations; Attach a report of the event and a copy of all examinations that were carried out, including the dates on which these examinations were performed. For laboratory tests, normal laboratory ranges must also be included.

It should be remembered that Serious Adverse Events (SAE) and Serious Adverse Drug Reactions (SADR) that are not documented in the Investigators' Brochure, or which occur in a more severe form than anticipated (i.e. they are 'unexpected'), are subject to rapid reporting to the Regulatory Authorities by the sponsor/promoter.

This also applies to reports from spontaneous sources and from any type of clinical or epidemiological investigation, independent of design or purpose. The source of the report (investigation, spontaneous, other) should always be specified.

Any queries concerning SAE or SADR reporting can be directed to the Safety Desk. All forms must be dated and signed by the responsible investigator or one of his/her authorized staff members.

All queries to the investigating center will be coordinated though the sponsor/promoter.

5.1 Primary Variables

Progression-Free Survival.

Progression-Free Survival (PFS) is the time from the date of randomization to the date of the first observation of documented disease progression or death due to any cause. Patients without tumor progression at the time of analysis were censored at their last date of tumor evaluation.

The Intention-to-treat (ITT) population is defined as the population of all enrolled patients. The activity (AP) and the safety population (SP) are considered as all patients who received, in each treatment group, at least one dose of treatment.

Descriptive statistics were reported for patients and tumors characteristics. Proportions were compared with chi-square or Fisher exact test as indicated, and continuous variables were compared by t-test or non parametric tests according to data distributions, providing 95% confidence intervals (95% CI).

Time to event data were analyzed using Kaplan-Meier curves, with 95% CI for median time and for each year of follow-up calculated with nonparametric methods.

Comparisons between the two treatment arms were performed using the log rank test, at a significance level of 10%. Unadjusted and adjusted hazard ratios (HR) were calculated using Cox proportional-hazard models, including or not the main known prognostic and predictive factors. Two-sided 95% CI for each HR were provided.

5.2 Secondary Variables

Quality of life.

Quality of life was measured with EORTC QLQ-C30 Version 3.0 and QLQ-BR23 (breast cancer specific) questionnaires.

Toxicity.

Each patient receiving at least one administration of study treatments will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and reports of AEs reported to the investigator by patients. Each patient will be assessed periodically for the development of any toxicity. Toxicity will be assessed according to the NCI CTCAE v5.0.
Time to treatment failure.

Time to treatment failure (TTF) is the time from randomization to treatment discontinuation for any reason, including disease progression, treatment toxicity, patient refusal or death.

Best objective response rate.

Best objective response rate (partial or complete response) was evaluated according to RECIST 1.1.

Duration of response.

Duration of response is the time from documentation of tumor response to disease progression.

Clinical benefit rate.

Clinical benefit rate (CBR) is the percentage of patients who achieved complete response, partial response or stable disease lasting longer than 24 weeks.

Overall Survival.

Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Time to Progression.

Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

Correlative biomarkers.

Correlative biomarkers were assessed on baseline tumor specimens (from primary tumor or metastatic biopsies) and blood samples collected at baseline and at different timepoints until evidence of disease progression.

Clinical endpoints were analysed with the methods described in the primary endpoint section. Time course of biomarkers levels and of quality of life scores were compared between the two groups by mixed effects models for repeated longitudinal data.

5.3   Measurements/Assessments

Efficacy and safety assessment were previously described.

Moreover, a correlative study was planned. As this study allows the use of different chemotherapeutic agents and regimens, at the discretion of the treating physician, biological assessments focused mainly on pathways involved in the response to chemotherapy in general, as opposed to features portending responsiveness to specific agents. Three main pathways are identified as important to this aim: p53, Rb, and p38/JNK MAPK pathways. The Cyclin D – CDK4/6 – Rb pathway is also crucial for responsiveness to CDK4/6 inhibitors.

6   Data Quality Assurance

The Investigators, by signing the protocol approval, agreed to perform the study in accordance with ICH Good Clinical Practice. The Investigator was required to ensure his compliance to the procedures required by the protocol with respect to the investigational drug schedule and visit schedule. The Investigator agreed to provide all information requested in the eCRF in an accurate and legible manner.
Study monitoring was directly organized by the CC. Periodic monitoring visits were conducted and the Principal Investigator provided access to the original records to permit verification of proper entry of data. At the completion of the study, all case report forms were reviewed by the Principal Investigator who signed them to state the accuracy of the data. Centers were monitored based on a risk analysis and eligibility for all patients was checked remotely before each randomization.
The monitor was trained by the study project manager, who was involved in the study development. Investigators and all staff personnel were initially trained by the study monitor or by the study project manager, during the site initiation visit. When new personnel was delegated on

site, the Principal Investigator trained them. Amendment 1.0 specific training was provided by the study project manager or the study monitor. All of the training was listed on a site specific staff training log.

All of the Principal Investigators involved were selected on the basis of their relevant experience on the subject, as described in the provided CV.

During the study, the project manager and the monitor ensured that the laboratory of each participating center send an up to date quality certificate and normal ranges list, to ensure standardisation and quality of laboratory values during the conduct of the trial.

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7 Data Management Procedures

The Investigator prepared and maintained adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug. Study personnel at each site entered data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) (appendix 5) when the information corresponding to that visit is available. Subjects were not identified by name in the study database or on any study documents to be collected by the Promoter (or designee), but were identified by a site number, subject number. eCRFs were completed through use of a Promoter-designated EDC system. Sites received training and had access to a manual for appropriate eCRF completion. eCRFs were submitted electronically to the Promoter and were handled in accordance with instructions from the Promoter. All eCRFs were completed by designated, trained site staff. eCRFs were reviewed and electronically signed and dated by the investigator or a designee. If a correction was required for an eCRF, the time and date stamped track the person entering or updating eCRF data and created an electronic audit trail. At the end of the study, the investigator received patient data for his or her site in a readable format on a compact disc that must be kept with the study records. Acknowledgement of receipt of the compact disc was required.

eCRF were designed by a multidisciplinary group including the Principal Investigator, the writing committee, the statistician and the datamanager, using standard coding dictionaries for the main clinical and treatment characteristics.

8 Statistical Considerations

8.1 Planned Statistical Methods

The statistical section of the study protocol detailed all the descriptive and inferential statistical analyses as following.

Analysis of primary endpoint.
The Intention-to-treat (ITT) population is defined as the population of all enrolled patients. The activity (AP) and the safety population (SP) are considered as all patients who received, in each treatment group, at least one dose of treatment. Descriptive statistics will be reported for patients and tumors characteristics. Proportions will be compared with chi-square or Fisher exact test as indicated, and continuous variables will be compared by t-test or non parametric tests according to data distributions, providing 95% confidence intervals (95% CI). Time to event data will be analyzed using Kaplan-Meier curves, with 95% CI for median time and for each year of follow-up calculated with nonparametric methods. Comparisons between the two treatment arms will be performed using the log rank test, at a significance level of 10%. Unadjusted and adjusted hazard

ratios (HR) will be calculated using Cox proportional-hazard models, including or not the main know prognostic and predictive factors. Two-sided 95% CI for each HR will be provided.

No stratification factor will be considered in the primary endpoint analysis.

The maximally selected rank statistics (MSRS) method was used to identify exploratory cut-offs to determine for each genomic signature/gene of interest high and low levels of expression, so to assess their association with PFS or OS. Since the study did not reach the required sample size, all the analyses presented in this report are intended to be hypothesis-generating, with a significance level set at $p<0.05$, without adjustments for multiplicity.

Baseline gene expression difference between the two treatment cohorts was assessed with two-class unpaired SAM analysis, with a false discovery rate (FDR)≤5%.

All statistical analyses were performed with R vers.3.6.1(21) and SPSS vers.24 (IBM Corp. Released 2016. IBM SPSS Statistics for MacOS, Version 24.0. Armonk, NY: IBM Corp.) for MacOSX. The study is registered with EudraCT number 2016-004107-31.

Analysis of secondary endpoints.
Clinical endpoints will be analyzed with the methods described in the primary endpoint section. Time course of biomarkers levels and of quality of life scores will be compared between the two groups by mixed effects models for repeated longitudinal data.

Interim analysis

Two interim analyses are planned after 105 and 128 patients are enrolled (i.e. after 70% and 85% of the maximum sample size, respectively). At each halt the PFS equality between the two arms against the superiority of arm B will be inferred by means of the log-rank test. The family wise error rate will be controlled by means of Lan and DeMets $\alpha\alpha$-spending function [Lan KG 1983] in order to preserve the nominal $\alpha = 0.10\alpha = 0.10$ significance level toward the final analysis.

Reporting and exclusions

Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first administration of chemotherapy Evaluation of response. Only those patients who have received at least one dose of therapy, and have had disease reevaluated, will be considered evaluable for response. These patients will have response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

Procedures to account for missing or spurious data

Missing data will be assumed to be missing completely at random for all analyses and no imputation will be done to estimate missing observations.

8.2 Determination of Sample Size

The primary endpoint is PFS, and the underlying statistical hypothesis is that the combination of chemotherapy plus endocrine therapy (AI or fulvestrant) (i.e. arm B) will improve PFS compared with concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant).

Assuming that the survival times follow an exponential distribution parameterized in terms of its expected value, the statistical hypothesis system is $H_0: \theta_A = \theta_B; \ H_1: \theta_B > \theta_A H_0: \theta_A = \theta_B; \ H_1: \theta_B > \theta_A$ at $\alpha = 0.10\alpha = 0.10$ significance level.

A maximum sample size of 150 patients will be recruited in a 23 month period for an estimated accrual rate of around 6.5 patients per month; they will be monitored for a 16 month follow-up. Initially, the patients will be randomly assigned according to block randomization (with blocks of size two) until two events (i.e. first disease progression or death whichever occurs first) are experienced in both arms. At this point, the allocation probabilities will be computed for the first time according to the Doubly-adaptive Biased Coin Design (DBCD) [Hu 2004] for time-to-event data [Zhang 2007] targeting the Neyman allocation rule (i.e. the one minimizing the total number of patients $n = n_A + n_B$ $n = n_A + n_B$). This randomized response adaptive procedure skews the allocation probabilities towards the arm with a longer estimated PFS allowing for a sensible ethical gain. Subsequently, these probabilities will be updated after 105 and 128 patients enter the study. At these time points, interim analyses will be performed in order to test the null hypothesis of equality of survival times [Zhu 2010]: whether will it be rejected the study is stopped and treatment B declared superior to A. In order to preserve the nominal significance level for the final analysis, Lan and DeMets $\alpha$$\alpha$-spending function [Lan KG 1983] will be adopted for opportunely correcting the intermediate significance levels. The introduction of the interim analyses further increases the ethical component of the design ensuring that no more than necessary patients will be enrolled. Table 1 shows the results of Monte Carlo simulations carried out under a growing median PFS in arm B –i.e. $_{med}\theta_B$ $_{med}\theta_B$ ranges from eight to 14 months– against a fixed eight month PFS in arm A in order to evaluate the operating characteristics with a maximum sample size of 150 patients. Under the null hypothesis (first row of Table 1) both the usual completely randomized design (CR) and the group sequential DBCD (G-DBCD) show a good control of type-I error rates. Further simulations, not displayed here for sake of brevity, confirmed that both designs maintain this paramount capability even for higher sample sizes and/or longer trial recruitment and duration. Furthermore, G-DBCD expected sample size (ESS) is close to the 150 patients CR fixed one while a good amount of patients is adaptively allocated ($\tau = 0.779$$\tau = 0.779$) in an almost balanced way ($\pi_B = 0.498$$\pi_B = 0.498$). This means that under the null hypothesis of equal PFSs, neither arm is preferred.

Increasing the median PFS of treatment B, allows to appreciate the considerable gain in terms of power induced by G-DBCD which allows the log-rank test to far better detect a longer PFS in arm B than under CR. Furthermore this ability is coupled with a progressively lower expected sample size compared to the fixed CR one. Less patients are adaptively assigned as the increase of $_{med}\theta_B$ $_{med}\theta_B$ results in a delay of the starting of the response adaptive randomization procedure since in average it takes more time to observe two events in arm B. On the other hand, the allocation proportion are increasingly skewed towards treatment B as it progressively shows a longer PFS. Let us now consider a median eight month PFS for arm A (Paloma 3 Study, supplementary material of Turner NC, NEJM 2018) and a 12 month one for arm B –i.e. $_{med}\theta_A = 8$$_{med}\theta_A = 8$ and $_{med}\theta_B = 12$ $_{med}\theta_B = 12$. Under this more likely scenario, the log-rank test coupled with G-DBCD is able to detect a four month median PFS increment in arm B with a considerably higher simulated power than under CR design: 0.911 versus 0.717, respectively. In addition, when compared to the fixed 150 patient sample size of the CR design, the G-DBCD shows a reduction of almost 29 patients in the expected sample size (ESS) due to the introduction of interim analyses allowing for early stopping for efficacy. This means that on average around 19% less individuals will be enrolled in the trial, stressing the considerable ethical gain of the proposed strategy. Concurrently, for the log-rank test to achieve the same power (0.911) as under G-DBCD analytical results shows that under CR design on average it needs around 184 total patients –i.e. an increase of around 52% patients with respect to the 121.259 ESS. Finally, under G-DBCD, on average, slightly more than 56% of the ESS is allocated to arm B ($\pi_B$$\pi_B$): a considerable ethical gain is again appreciable.

Table 1. Results of the Monte Carlo simulation for log-rank test coupled with the complete randomization (CR) and group sequential DBCD (G-DBCD) assuming a median PFS of eight months for arm A and an increasing one for the experimental one ($_{med}\theta_B$ $_{med}\theta_B$): power of the two strategies and expected sample size (ESS), allocations proportions to the experimental ($\pi_B$$\pi_B$) and percentages of patients adaptively allocated

to arm B ($\tau\tau$) under G-DBCD.

$_{med}\theta_B$	power		ESS	π_B	τ
	CR	G-DBCD			
8.0	0.084	0.089	147.014	0.498	0.779
9.0	0.205	0.458	138.317	0.509	0.758
10.0	0.384	0.741	129.897	0.529	0.732
11.0	0.564	0.841	125.304	0.547	0.713
12.0	0.717	0.911	121.259	0.563	0.695
13.0	0.829	0.948	117.939	0.580	0.678
14.0	0.908	0.972	115.130	0.595	0.662

Figure 1. Power of the nonparametric test under complete randomization (CR) and group sequential DBCD (G-DBCD).

All patients fulfilling the eligibility criteria will be randomized by the Biostatistics and Clinical Trial Unit of the CC, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST). No blinding is planned.

The study duration will be 39 months; 23 months of accrual and 16 months of follow-up on the last participant enrolled.

9 Changes in the Conduct of the Study or Planned Analysis

Changes in the conduction of the study or in the planning of the analysis were performed as a function of the first amendment of the study. The protocol amendment, as already described, was approved by Ethical Committee of IRST and accepted by each participating center.

10 Results

10.1 Study Subjects/Patients

a) Disposition of Subjects

A total of 52 patients from 10 Italian institutions were assessed for eligibility. Of these, 49 were ultimately randomized, with 17 (34.7%) being assigned to arm A, and 32 (65.3%) to arm B. See Figure 1 for disposition of analysis population.

Figure 1. KENDO CONSORT diagram
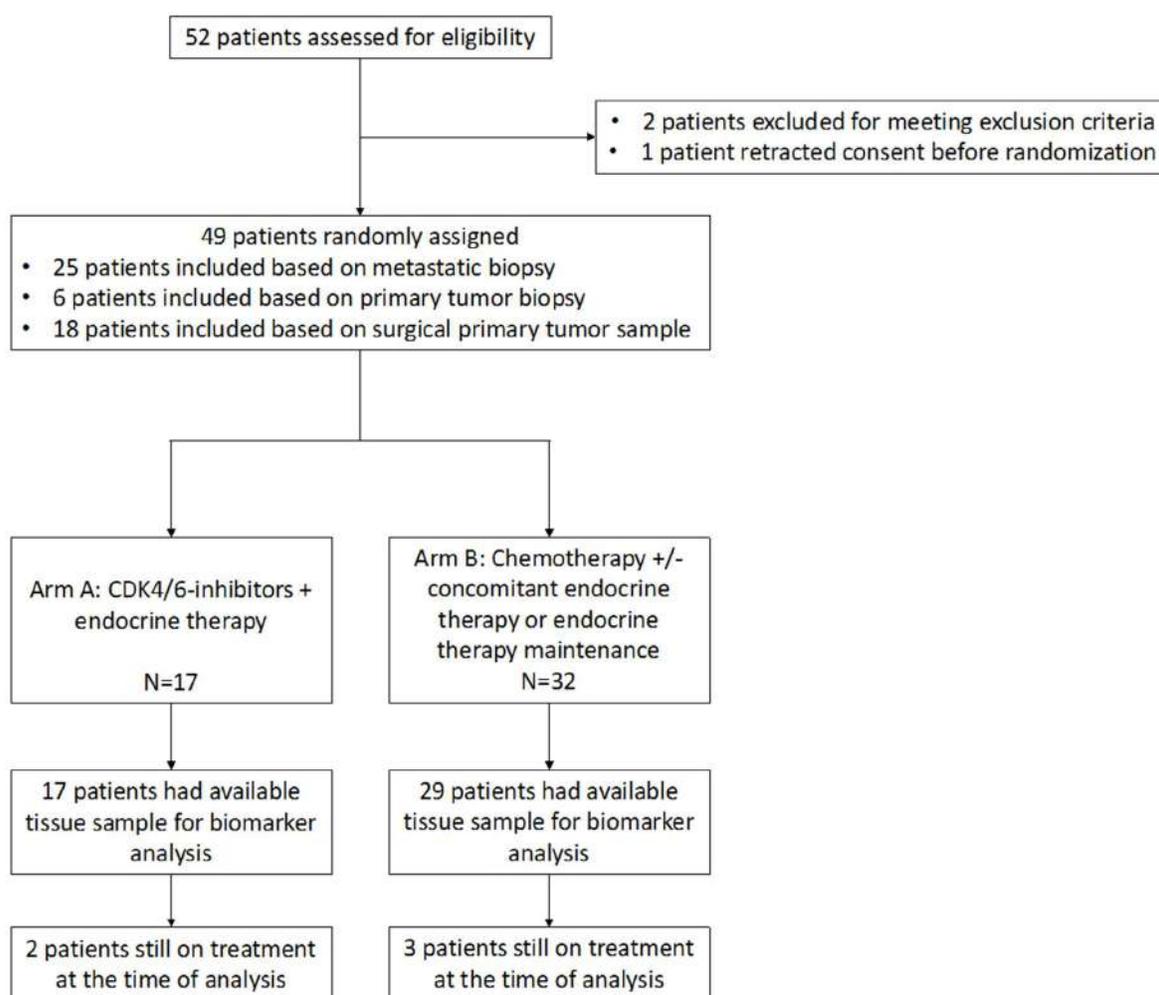
b) Protocol Deviations

Out of 49 ITT evaluable patients who started therapy, no protocol deviations were observed for entry criteria violation, delay or reduction or incorrect study treatment or doses.

10.2 Efficacy Evaluation

a) Data Sets analyzed

From July 2017 to November 2020, 49 subjects were randomized from 10 active participating centers, 17 in Arm A and 32 in Arm B. All patients assigned to a specific treatment arm received at least one dose of the preplanned medication, were included in the efficacy analysis for the primary response variable according to protocol ITT population definition.

b) Demographic and Other Baseline Characteristics

Demographic, clinical and tumor characteristics of 49 ITT patients at baseline are listed in Table 1. No significant differences at baseline were observed between the 2 treatment arms in terms of main clinicopathological and tumor molecular features. No subgroup analyses are performed.

Table 1. Population characteristics

POPULATION DEMOGRAPHICS		ARM A		ARM B		P
		N	%	N	%	
		17	34.7	32	65.3	
Age at randomization						
	Median	64	-	62	-	0.366
	IQR	55 - 67		50 - 69		
Menopausal status at randomization						
	Premenopausal	2	12.5	6	21.4	0.460
	Postmenopausal	14	87.5	22	78.6	
	Overall	16	94.1	28	87.5	

Metastatic status at randomization						
	De novo	3	17.6	12	37.5	0.151
	Relapsed	14	82.4	20	62.5	
Neo/adjuvant CT in relapsed patients						
	Yes	8	57.1	8	38.1	0.268
	No*	6	42.9	13	61.9	
	Overall	14	82.4	21	65.6	
Neo/adjuvant ET in relapsed patients						
	Yes	14	82.4	26	81.3	0.924
	No*	3	17.6	6	18.8	
Number of metastatic sites						
	< 3	2	11.7	2	6.3	0.502
	≥ 3	15	88.2	30	93.7	
Tissue sample for randomization						
	Primary tissue	7	41.2	17	53.1	0.426
	Metastatic tissue	10	58.8	15	46.9	

Metastatic spread

Bone-only	3	17.6	6	18.8	0.995
Non visceral	7	41.2	13	40.6	
Visceral	7	41.2	13	40.6	

Endocrine sensitivity

Sensitive	13	76.5	29	90.6	0.178
Primary Resistant	4	23.5	3	9.4	
Secondary Resistant	0	0.0	0	0.0	

Estrogen Receptor (%)

ER 10-50%	2	11.8	3	9.4	0.793
ER>50%	15	88.2	29	90.6	

Progesterone Receptor (%)

<20	6	35.3	14	43.8	0.566
≥20	11	64.7	18	56.3	

KI67 (%)

<20	4	30.8	7	31.8	0.949
≥20	9	69.2	15	68.2	

Overall	13	76.5	22	68.8	

PAM50 Intrinsic Subtype

Luminal A	3	17.6	7	24.1	0.721
Luminal B	6	35.3	10	34.5	
HER2E	1	5.9	4	13.8	
Basal-like	2	11.8	1	3.4	
Normal-like	5	29.4	7	24.1	
Overall	17	100.0	29	90.6	

ROR-P

Low	3	17.6	5	17.2	0.988
Intermediate	9	52.9	16	55.2	
High	5	29.4	8	27.6	
Overall	17	100.0	29	90.6	

Tumor infiltrating lymphocytes (%)

Median	3	-	4.5	-	0.129
IQR	1 - 5	-	2 - 12.8	-	

TLS[#] (w/wo germinal centers)

Yes	2	12.5	14	50.0	0.583
No	14	87.5	14	50.0	
Overall	16	94.1	28	87.5	

Immune pattern

Inflamed	2	12.5	7	25.0	0.295
Excluded	0	0.0	2	7.1	
Desert	14	87.5	19	67.9	
Overall	16	94.1	28	87.5	

Legend. Arm A: CDK4/6-inhibitor-based arm; Arm B: chemotherapy-based arm; ER: estrogen receptor; IQR: interquartile range; ROR-P: PAM50 risk of relapse score including subtypes and proliferation signatures; *: includes patients with de novo metastatic disease. *Overall* identifies the number of patients per arm for whom the information is available for a specific variable..

c) Treatment Compliance

All 49 patients received at least one cycle of treatment. In arm A, CT alone was only administered in 6.3% patients, while in most cases (62.5%) patients stopped CT after 4-6 months and received ET maintenance. Concomitant and/or maintenance ET consisted exclusively in an AI. The majority of patients in arm B received capecitabine-based regimens (68.8%) and none received a regimen containing both anthracycline and taxanes. The only taxane administered was paclitaxel in weekly schedule. The treatments administered are fully reported in Table 2.

Table 2. Study treatments

STUDY TREATMENT DETAILS	STUDY POPULATION

	N	%
	49	100.0
Endocrine therapy + CDK4/6-inhibitors	17	34.7
CDK4/6-inhibitor		
Palbociclib	7	41.2
Ribociclib	6	35.3
Abemaciclib	4	23.5
Endocrine partner		
Aromatase inhibitor	10	58.8
Fulvestrant	7	41.2
Chemotherapy (+/- endocrine therapy)	32	65.3
Strategy		
Concomitant ET + maintenance	10	31.3
Only maintenance ET	20	62.5
Chemotherapy alone	2	6.3
CT type		
Anthracycline-based	6	18.8

Taxane-based	4	12.5
Capecitabine monotherapy	14	43.8
Capecitabine + vinorelbine	8	25.0

Legend. CT: chemotherapy; ET: endocrine therapy; taxane-based regimens consisted in intravenous (i.v.) weekly (qw) paclitaxel; anthracycline-based regimens include: i.v. liposomal doxorubicin + cyclophosphamide every 3 weeks (q3w) and standard doxorubicin + cyclophosphamide q3w.

d) Efficacy Results

Primary endpoint and other efficacy and activity endpoints
At a median follow-up of 35.2 months (95% CI 30.6-43.7), 15 (88.2%) patients in arm A and 29 (90.6%) patients in arm B had experienced a PFS event, with a median PFS (mPFS) for arm A of 19.9 months (95% CI 9.0-30.6) and mPFS for arm B of 11.2 months (95% CI 7.7-15.4) (B vs. A HR: 1.41, 95% CI 0.75-2.64, p=0.289). The median OS (mOS) for arm A was not reached (NR) at the time of analysis (95% CI 24.4 months-not estimable[NE]), while for arm B it was 30.6 months (95% CI 24.5-NE), although the difference was not statistically significant (arm B vs. A HR=1.66, 95% CI 0.66-4.19, p=0.283) (Figure 2). Four deaths were observed during the study directly associated to tumor progression, 1 in arm A and 3 in arm B. Cross-over upon PD concerned 4 patients in total, 3 from the CT arm and 1 from the CDK4/6-inhibitors arm.

Figure 2. PFS and OS according to treatment arm, PAM50 IS and ROR-P
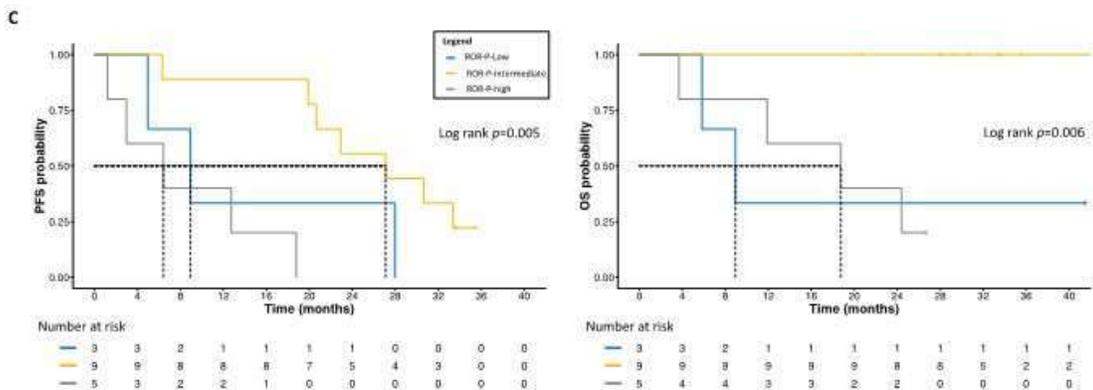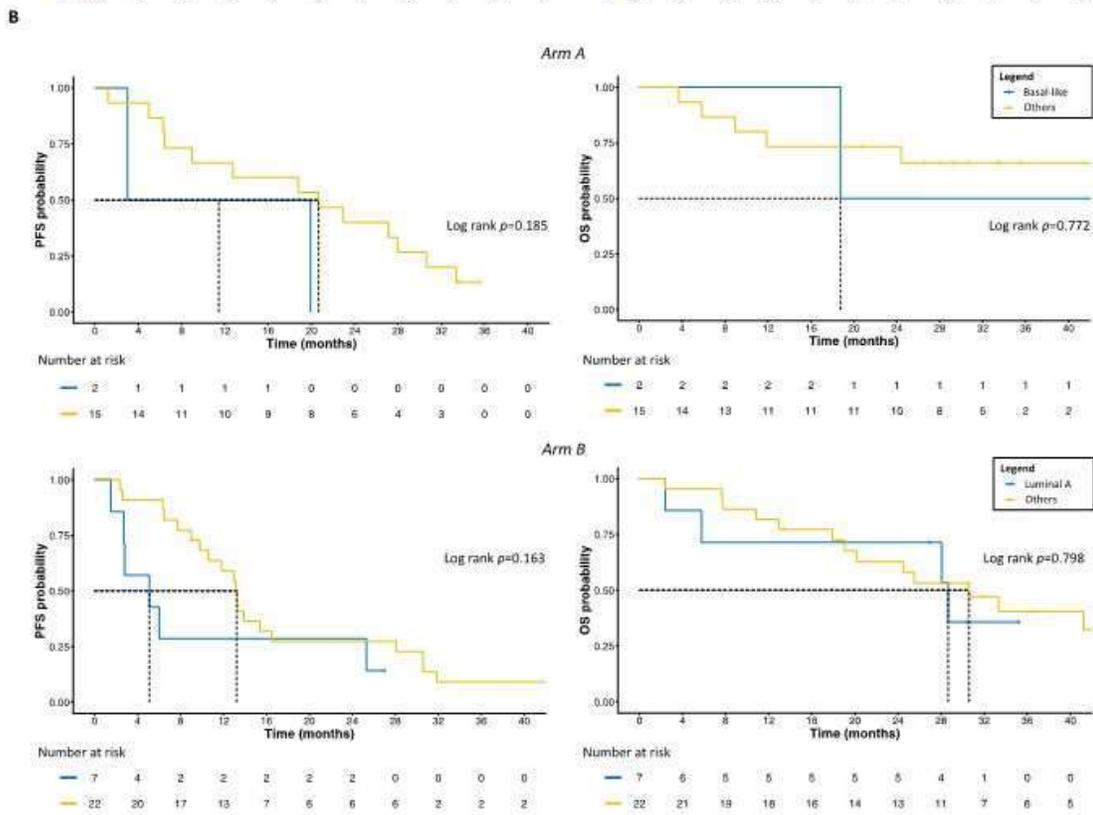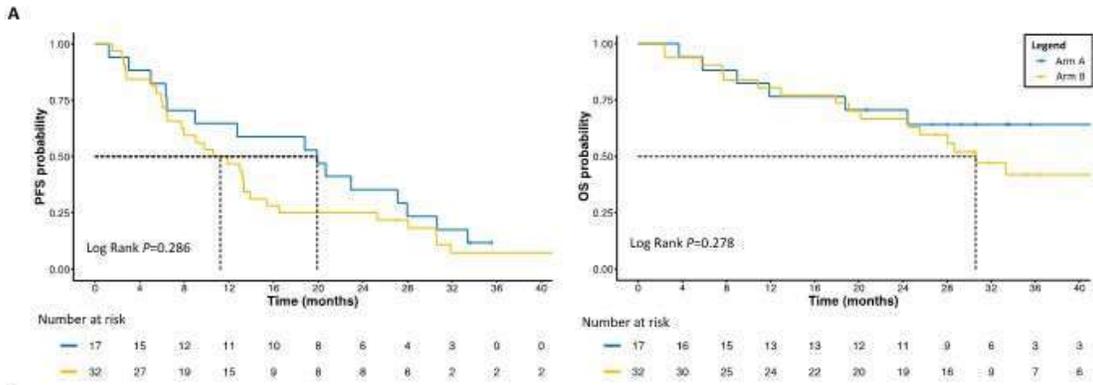
Legend. A: PFS and OS according to treatment arm B: PFS and OS in arm A and B based on PAM50 IS; C: PFS and OS in arm A, according to ROR-P category. PFS: progression-free survival; OS: overall survival; IS: intrinsic subtypes.

Safety

No patient stopped the treatment due to toxicity, withdrawal or other causes unrelated to disease progression or death. No standard clinicopathological variables were found to be significantly associated with PFS and OS.

The most common (≥5%) grade 3–4 AEs in both arms was neutropenia (41.2% in arm A and 22% in arm B, respectively). A detailed report of AEs can be found in Table 3. No patient stopped study treatment because of toxicity. Among patients treated in arm A, 2 of them (12%) required a dose reduction, instead in the arm B, 10 (31%) patients. No serious adverse events have been reported. Overall, the safety profile was consistent with previous literature and manageable, without novel unexpected safety concerns. CT with concomitant ET led to a slight increase in AEs due to the addition of ET-related toxicities.

Table3. Summary of adverse event

Summary of adverse event				
	ARM A (n= 17)		ARM B (n= 32)	
Adverse event according to CTCAE v5.0	G1/G2	G3/G4	G1/G2	G3/G4
Anemia	4 (23.5)	1 (5.8)	4 (12.5)	0
Asthenia/Fatigue	**10(58.8)**	1 (5.8)	**18 (56.2)**	0
Diarrhea	6(35.3)	0	8 (25.0)	0
Fever	2 (11.8)	0	7 (22.0)	0
Alopecia	2 (11.8)	0	0	0
Nausea/Vomiting	3 (17.6)	1 (5.8)	8 (25.0)	0

Neutropenia	4 (23.5)	**7 (41.2)**	4 (12.5)	**7 (22.0)**
Constipation	0	0	5 (15.6)	0
Peripheral neuropathy	0	0	5(15.6)	0

Although not significant (*p*=0.260), ORR with CDK4/6-inhibitors+ET doubled the ORR observed in the CT arm (31.3% vs. 16.7%), with numerically longer DOR (12.6 vs. 7.0 months, *p*=0.368). CBR was similar between the 2 arms (Table 4).

Table 4. Tumor responses according to treatment arm in assessable patients

RESPONSE TYPE AND DURATION	Arm A			Arm B			*P*
	N	%	95%CI	N	%	95%CI	
	16	94.1	-	30	93.8	-	
CR	1	6.3	-	2	6.7	-	0.702*
PR	4	25.0	-	3	10.0	-	
SD>3months	8	50.0	-	19	63.3	-	
SD<3months	1	6.3	-	1	3.3	-	
PD	2	12.5	-	5	16.7	-	
ORR	5/16	31.3	11.0-58.7%	5/30	16.7	5.6-34.7%	0.260[#]

CBR	13/16	81.3	54.4-96.0%	24/30	80.0	61.4-92.3%	0.720[#]
DOR	12.6 months	-	3.9 months - NE	7.0 months	-	4.2-12.2 months	0.368[§]

Legend. CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; ORR: overall response rate; CBR: clinical benefit rate; CI: confidence interval; NE: not estimable; DOR: duration of response; *: *p* value for chi square test; #: *p* value for univariate logistic regression; §: *p* value for log rank test.

Genomic correlative biomarker analyses

When we applied the PAM50 algorithm for intrinsic subtyping in baseline tumor samples, overall, 26 (56.5%) tumors were found to be Luminal and 20 (43.5%) were non-Luminal. The most prevalent PAM50 risk-of-relapse (ROR)-P score category was ROR-intermediate (54.3%). No significant gene expression differences (all FDR>5%) and no differences in the distribution of PAM50 intrinsic subtypes (IS) and ROR-P categories were observed between the 2 cohorts (Table 5).

Table 5. Full list of genes included in the Breast Cancer 360™ panel and signatures of interest

GENES AND SIGNATURES									
ABCA8	*CAV1*	*CMKLR1*	*EYA1*	*HBB*	*JAG1*	*MTOR*	*PLA2G4A*	*SFN*	*TMPRSS4*
ABCF1	*CBLC*	*CNTFR*	*EYA2*	*HDAC1*	*JAG2*	*MUC1*	*PLA2G4F*	*SFRP1*	*TNF*
ACTR3B	*CCL2*	*COL11A1*	*EYA4*	*HDAC10*	*JAK1*	*MUS81*	*PLAT*	*SFRP2*	*TNFAIP6*

ACVR1B	CCL21	COL27A1	F3	HDAC11	JAK2	MYBL2	PLCB1	SFRP4	TNFSF10
ACVR1C	CCL3L1	COL2A1	FAM124B	HDAC2	JAK3	MYC	PLCB4	SHC2	TNKS
ACVRL1	CCL4	COL4A6	FAM198B	HDAC5	JAM2	MYCN	PLCE1	SHC4	TNKS2
ADAM12	CCL5	COL6A3	FAM214A	HDAC6	JCAD	MYCT1	PLD1	SHE	TNN
ADCY9	CCL7	COL7A1	FAM83D	HDC	JUN	NASP	PMS2	SHMT2	TOP2A
ADD1	CCL8	COL9A3	FANCF	HEG1	KAT2B	NAT1	POLD1	SIDT1	TP53
ADM	CCNA1	COLEC12	FAP	HELLS	KCNB1	NCAM1	POLQ	SIGIRR	TPSAB1
AGR2	CCNA2	COMP	FBN1	HEMK1	KDR	NCAPH2	POLR2A	SIX1	TRIP13
AGT	CCNB1	CPA3	FGF1	HES1	KIAA0040	NDP	POPDC3	SKA3	TSPAN1
AGTR1	CCND1	CREBBP	FGF10	HGF	KIF11	NEIL1	PPARG	SKP1	TSPAN7
AKT3	CCND2	CRYAB	FGF12	HIF1A	KIF14	NEIL2	PPARGC1A	SKP2	TTK
ALDH1A1	CCNE1	CSF3R	FGF13	HIST1H1C	KIF23	NEIL3	PPP2CB	SLC2A1	TTYH1
ALDOA	CCNE2	CTSW	FGF18	HIST1H2BH	KIF2C	NEO1	PPP2R1A	SLC39A6	TUBA44A
ANGPT1	CCR1	CXADR	FGF2	HIST1H3H	KIFC1	NETO2	PPP2R2C	SLC44A4	TWIST1
ANLN	CCR2	CXCL10	FGF7	HIST3H2BB	KIT	NFATC1	PRC1	SLPI	TWIST2
ANXA9	CCR5	CXCL12	FGF9	HK2	KLRK1	NFKBIZ	PREP	SMAD1	TYK2
APH1B	CD163	CXCL13	FGFR2	HLA-A	KNTC2	NGFR	PRF1	SMAD3	TYMP
APOD	CD19	CXCL5	FGFR3	HLA-B	KRT14	NKG7	PRKAA2	SMAD4	TYMS

APOE	CD1E	CXCL8	FGFR4	HLA-C	KRT17	NOD2	PRKACA	SMAD5	UBB
AR	CD24	CXCL9	FGL2	HLA-DMA	KRT5	NOTCH1	PRKACB	SMC1B	UBE2C
AREG	CD27	CXCR6	FHL1	HLA-DMB	KRT6B	NOTCH2	PRKCA	SMO	UBE2T
ARID1A	CD274	CXorf36	FLI1	HLA-DOB	KRT7	NOTCH3	PRKCB	SMURF2	VCAN
ARNT2	CD276	CXXC5	FLNC	HLA-DPA1	LAD1	NPEPPS	PRKDC	SNAI1	VEGFA
ASPM	CD34	CYBB	FLRT3	HLA-DPB1	LAG3	NPR1	PRKX	SNAI2	VEGFD
ASPN	CD36	CYP4F3	FLT3	HLA-DQA1	LAMA3	NR4A1	PRLR	SOCS1	VIM
ATAD2	CD44	DCN	FNBP1	HLA-DQB1	LAMB3	NR4A3	PROM1	SOCS2	VIT
ATM	CD68	DDB2	FOS	HLA-DRA	LAMC2	NRCAM	PSAT1	SOCS3	WDR77
ATP10B	CD84	DDR2	FOSL1	HLA-DRB1	LEF1	NRDE2	PSMB10	SOX10	WEE1
AURKA	CD8A	DDX39A	FOXA1	HLA-E	LEFTY2	NRXN1	PSMB7	SOX17	WIF1
AURKB	CD8B	DEPDC1	FOXC1	HMGA1	LEMD1	NRXN3	PSMB9	SOX2	WNT10A
AXIN1	CDC14A	DHRS2	FOXC2	HNF1A	LEP	NSD1	PTCH1	SOX9	WNT11
AXIN2	CDC14B	DKK1	FOXM1	HOXA5	LEPR	NSD3	PTEN	SP1	WNT2
B3GNT3	CDC20	DKK2	FOXP3	HOXA7	LFNG	NTRK2	PTGDS	SPC25	WNT4
BAD	CDC25A	DLGAP5	FREM2	HOXA9	LIF	NUDT1	PTGER3	SPDEF	WNT5A
BAG1	CDC25B	DLL1	FST	HOXB13	LIFR	NUMBL	PTGS2	SPN	WNT5B
BAIAP2L1	CDC25C	DLL3	FSTL1	HOXB3	LINC02381	NUPR1	PTTG1	SPP1	WNT6
BAIAP3	CDC6	DLL4	FSTL3	HSPA2	LPL	OAS3	PUM1	SPRY1	WNT7B

BAMBI	*CDC7*	*DNAJC12*	*FUT3*	*IBSP*	*LRP2*	*OAZ1*	*PYCARD*	*SPRY2*	*WRN*
BAX	*CDCA1*	*DPT*	*FXYD3*	*ICAM1*	*LRRC32*	*OCLN*	*RAC2*	*SPRY4*	*WT1*
BBC3	*CDCA5*	*DSC2*	*FZD10*	*ID1*	*LTB*	*OGN*	*RAC3*	*SRPX*	*XRCC2*
BBOX1	*CDCA7L*	*DTX1*	*FZD7*	*ID2*	*LTBP1*	*OLFML2B*	*RAD51*	*ST6GALNAC2*	*XRCC3*
BCAS1	*CDCA8*	*DTX3*	*FZD8*	*ID4*	*MAD2L1*	*ORC6L*	*RAD51C*	*STAT1*	*ZBTB16*
BCL11A	*CDH1*	*DTX4*	*FZD9*	*IDO1*	*MAF*	*PALB2*	*RAD52*	*STC1*	*ZEB1*
BCL2	*CDH2*	*DUSP4*	*G6PD*	*IFT140*	*MAML2*	*PALMD*	*RAD54L*	*STK11IP*	*ZEB2*
BCL2A1	*CDH3*	*DUSP6*	*GABRP*	*IGF1*	*MAP2K4*	*PARP1*	*RARRES3*	*SUV39H2*	*ZFPM2*
BCL2L1	*CDH5*	*E2F1*	*GADD45A*	*IGF1R*	*MAP3K12*	*PARP2*	*RASAL1*	*SYTL4*	*ZFYVE9*
BCL6B	*CDK1*	*E2F5*	*GADD45B*	*IKZF3*	*MAPK1*	*PARP4*	*RASGRF1*	*TAP1*	*ZIC2*
BDNF	*CDK4*	*ECM2*	*GADD45G*	*IL10RA*	*MAPK10*	*PAX5*	*RASGRF2*	*TAP2*	*ZNF205*
BIRC5	*CDK6*	*EDN1*	*GAS1*	*IL11RA*	*MAPK3*	*PAX8*	*RASGRP1*	*TAPBP*	PAM50 Luminal signature
BLM	*CDKN1A*	*EDNRB*	*GATA3*	*IL12RB2*	*MAPK8IP2*	*PBX3*	*RB1*	*TBC1D10B*	PAM50 HER2 signature
BLVRA	*CDKN1B*	*EFNA3*	*GATA4*	*IL13RA1*	*MAPT*	*PCK1*	*RBL1*	*TBC1D9*	PAM50 Basal signature
BMP2	*CDKN1C*	*EFNA5*	*GDF15*	*IL1B*	*MARCO*	*PCNA*	*RBL2*	*TBP*	PAM50 Proliferation signature
BMP4	*CDKN2A*	*EGF*	*GDF5*	*IL1R2*	*MCM2*	*PDCD1*	*RBX1*	*TBX1*	Adhesion and Migration
BMP5	*CDKN2B*	*EGFR*	*GGH*	*IL1RN*	*MCM3*	*PDCD1LG2*	*RELN*	*TCEAL1*	Angiogenesis
BMP6	*CDKN2C*	*EGLN2*	*GHR*	*IL20RA*	*MDM2*	*PDE9A*	*RFC4*	*TCF4*	Antigen Presentation

BMP7	CDKN2D	EGLN3	GJB2	IL20RB	MED1	PDGFB	RNASE2	TCF7L1	Apoptosis
BMP8A	CDKN3	EIF2AK3	GLI3	IL22RA2	MELK	PDGFRA	RNF103	TEK	Cytokine/Chemokine Signaling
BMPR1A	CEACAM5	EIF3B	GNG4	IL24	MEOX2	PDGFRB	ROBO4	TFDP1	DNA Damage Repair
BMPR1B	CEACAM6	EIF4E2	GNLY	IL2RA	MET	PDK4	ROCK1	TFF1	EMT
BMPR2	CENPF	EIF4EBP1	GPC4	IL2RB	MFNG	PECAM1	ROCK2	TFF3	Epigenetic regulation
BNC2	CEP55	ELF3	GPR160	IL3RA	MIA	PFDN2	RORA	TFRC	Hedgehog
BNIP3	CETN2	ELK3	GPX3	IL4R	MIS18A	PGK1	RORB	TGFB1	Immune Infiltration
BORCS7	CFD	ELOVL2	GRB2	IL6	MKI67	PGR	RPS6KA5	TGFB2	JAK STAT
BRCA1	CHAD	EMCN	GRB7	IL6R	MLH1	PHGDH	RPS6KB1	TGFB3	MAPK
BRCA2	CHEK2	ENO1	GREM1	IL7R	MLLT3	PIK3CA	RPS6KB2	TGFBR2	Notch
BTG2	CHI3L1	ENPP2	GRIA3	INHBA	MLPH	PIK3CD	RRM2	THBS1	PI3K
C5orf38	CHIT1	EP300	GRIN1	INHBB	MME	PIK3CG	RUNX3	THBS2	Stromal Markers
CA12	CHRNA5	EPAS1	GRIN2A	IRF6	MMP11	PIK3R1	S100A14	THBS4	TGF beta
CACNA1D	CKB	ERBB2	GSK3B	IRX1	MMP14	PIK3R2	S100A7	THY1	Transcriptional Misregulation
CACNA1H	CKMT1A	ERBB4	GTF2H2	ISG15	MMP3	PIK3R3	S1PR1	TIE1	Tumor Metabolism
CACNA2D1	CKS1B	ERCC1	GUSB	ISM1	MMP7	PIK3R5	SCARA5	TIGIT	Wnt
CACNA2D3	CLDN1	EREG	GZMA	ITGA6	MMP9	PIM1	SCUBE2	TIMP4	CD8 T cells
CACNG1	CLDN3	ESPL1	GZMB	ITGAV	MMRN2	PIP	SDHA	TLE3	Cytotoxic cells
CACNG4	CLDN4	ESR1	GZMH	ITGB1	MRE11	PKMYT1	SELE	TLR4	Macrophages

CACNG6	*CLDN7*	*ETV4*	*GZMM*	*ITGB3*	*MS4A2*	*PLA1A*	*SERBP1*	*TLX1*	Mastcells
CALML5	*CLEC14A*	*ETV7*	*HAPLN1*	*ITGB6*	*MSR1*	*PLA2G2A*	*SERPINB5*	*TMEM45B*	ER signaling
CAMK2B	*CLEC5A*	*EXO1*	*HAS1*	*ITPR1*	*MT1G*	*PLA2G3*	*SERPINH1*	*TMPRSS2*	Triple Negative Biology

Legend. EMT: epithelial-to-mesenchymal transition.

In general, Luminal vs. non-Luminal tumors were not dissimilar in terms of PFS and OS (Figure 3).

Figure 3. PFS and OS according to PAM50 IS

A

PFS probability — Log rank *p*=1.0

OS probability — Log rank *p*=0.8

Legend
— Luminal
— Non-Luminal

B

PFS probability — Log rank *p*=0.8

OS probability — Log rank *p*=0.8

Legend
— Arm A
— Arm B

C

PFS probability — Log rank *p*=0.3

OS probability — Log rank *p*=0.1

Legend
— Arm A
— Arm B

However, when dissecting by treatment arm, we noticed that under CDK4/6 inhibition and ET, Basal-like tumors showed the numerically worse PFS (median 11.4 months, 95% CI 3.00-NE) and OS (median 18.8 months, 95% CI 18.8-NE), compared to the other subtypes (mPFS 20.7 months, 95% CI 9.00-33.4; mOS: NR, 95% CI 24.4-NE) (Figure 2). Conversely, in the CT arm, Luminal A tumors performed numerically worse in PFS (median 5.1 months, 95% CI 2.7-NE) than other IS (median 13.2 months, 95% CI 10.6-28.1). Nevertheless, OS was similar between Luminal A (median 28.7 months, 95% CI 5.8-NE) and other IS (median 30.6 months, 95% CI 20.2-NE) (Figure 2). When comparing the performance of each IS according to treatment arm, we noticed that Luminal B and HER2E tumors showed very similar PFS and OS (not shown). Differently, Luminal A, if treated with CT rather than CDK4/6-inhibitors+ET, showed numerically worse PFS (mPFS 5.1 [95% CI 2.7-NE] vs. 20.7 months [95% CI 6.3-NE]) and OS (mOS 28.7 [95% CI 5.8-NE] vs. NR [95% CI NE-NE]), whereas Basal-like showed the opposite (mPFS 13.3 [95% CI NE-NE] vs. 11.4 months [3.0-NE]; mOS 41.2 [95% CI NE-NE] vs. 18.8 months [95% CI 18.8-NE]) (Figure 3). To note, one of the two Basal-like tumors in arm A was associated to a germline *BRCA2* mutation and showed significantly higher PFS and OS than the other Basal-like in the same arm (mPFS 19.9 vs. 3.0 months and mOS 47.4 vs. 18.8 months, respectively).

In terms of ROR-P, despite not being associated to PFS and OS as continuous variable ($p>0.05$ in all cases), in arm A, ROR-P-intermediate vs. ROR-P-low/-high tumors showed significantly better PFS (HR=0.21, 95% CI 0.06-0.67, $p=0.009$) and OS (HR<1.0, 95% CI not evaluable, log rank $p=0.001$) (Figure 2). In arm B, there were no significant differences in PFS and OS, nor specific trends, based on ROR-P (log rank $p=0.558$ and $p=0.839$, respectively).

We performed multiple univariate survival analyses to evaluate the association with PFS and OS of all genes and signatures of interest included in the BC360 panel. Overall, only 42 (6.7%) genes were associated to PFS and/or OS in at least one of the 2 treatment cohorts. Genes associated to several signaling pathways usually involved in breast cancer cells' survival and proliferation (e.g. PI3K, MAPK, Hedgehog and Wnt pathways) were found to be associated to worse outcome when upregulated in most cases. Higher levels of expression of genes involved in proliferation, adhesion and migration or EMT were mostly associated to worse PFS and/or OS. Genes included in immunological signatures of antigen presentation and immune infiltration were associated generally to better outcomes. Most genes relevant for ER signaling or Luminal biology were associated to better outcomes, as well. Genes associated to biological processes favoring cancer development when suppressed, were usually associated to better outcomes if highly expressed (e.g. genes involved in DNA damage repair, TGF-beta pathway, *TP53*). *CD24* was the only gene showing a significant association with PFS in both arm A (HR=1.50, $p=0.040$) and B (HR=1.46, $p=0.025$).

We subsequently calculated several biologic signatures of interest associated with biological pathways or BC biology (Table 5). When considered as continuous variables, none of them was associated to PFS, nor OS. When dichotomizing them by using the MSRS method, we observed that in the CDK4/6-inhibitors-treated cohort, higher levels of Hedgehog, JAK-STAT, MAPK, PI3K and Wnt pathway signatures, PAM50 HER2 and proliferation and ER signaling signatures

were significantly associated to better PFS and OS (except the HER2 and Wnt signatures) (Figure 4).

Figure 4. Significant associations with survival outcomes of selected PAM50 and BC360 signatures in arm A
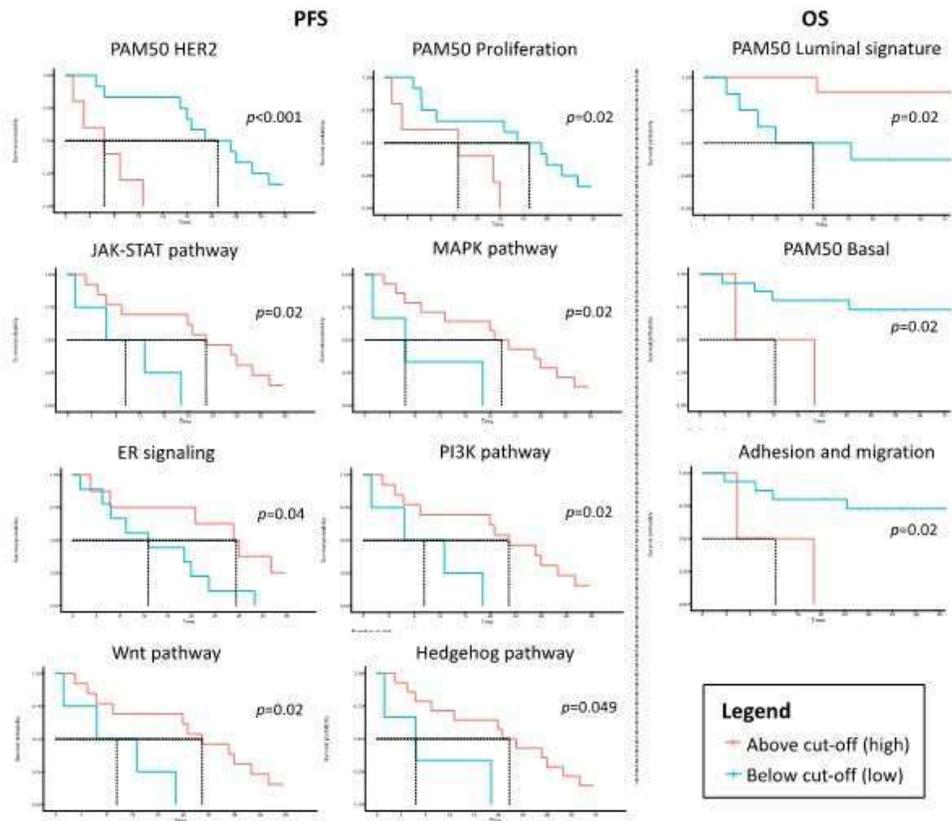
PFS

OS

PAM50 HER2

p<0.001

PAM50 Proliferation

p=0.02

PAM50 Luminal signature

p=0.02

JAK-STAT pathway

p=0.02

MAPK pathway

p=0.02

PAM50 Basal

p=0.02

ER signaling

p=0.04

PI3K pathway

p=0.02

Adhesion and migration

p=0.02

Wnt pathway

p=0.02

Hedgehog pathway

p=0.049

Legend
— Above cut-off (high)
— Below cut-off (low)

Higher levels of adhesion, migration and PAM50 Basal signatures were significantly associated to worse OS, while higher levels of the PAM50 Luminal signature provided better OS (Figure 4). Differently, in arm B, only higher levels of the PAM50 Luminal and Notch pathway signatures were significantly associated to better PFS (p=0.02 and p=0.04), with the former also associated to better OS (p=0.02) (not shown).

Immune biomarker analyses

We calculated several immunologic signatures of interest tracking immune response processes or specific immune cell types (Table 5). None of those was significantly associated to PFS, nor OS, as continuous variable. When dichotomizing them according to the MSRS method, we observed that higher levels of the TGF-beta signature were significantly associated to better PFS and OS in arm A and PFS in arm B, higher levels of a cytokine/chemokine signature were associated to better PFS in both arms, and higher levels of a mast cells signature was associated to worse OS in both arms. Higher levels of a macrophages signature were also associated to worse OS, but only in arm B. In the same arm, higher levels of an immune infiltration signature and cytotoxic cells were associated to better PFS, higher levels of a CD8 T cell signature were associated to better PFS and OS and higher levels of an antigen presentation signature were associated to better OS, as well (Supplementary figure 5). In arm A, higher levels of the antigen presentation (p=0.072) and immune infiltration (p=0.072) signatures showed a trend towards better OS, whereas higher levels of CD8 T cells (p=0.07) and cytotoxic cells (p=0.06) signatures showed favorable trends in PFS (not shown).

We further assessed the presence of TILs and TLS (with or without germinal center), as well as the pattern of immune infiltration at IHC, and assessed potential associations with PFS and OS.

After applying the MSRS method, we observed that higher TILs levels were significantly associated to better PFS (*p*=0.003) and OS (*p*=0.005) in arm A, but not in arm B (Figure 5).

Figure 5. Significant associations with PFS/OS of TILs and immune signatures
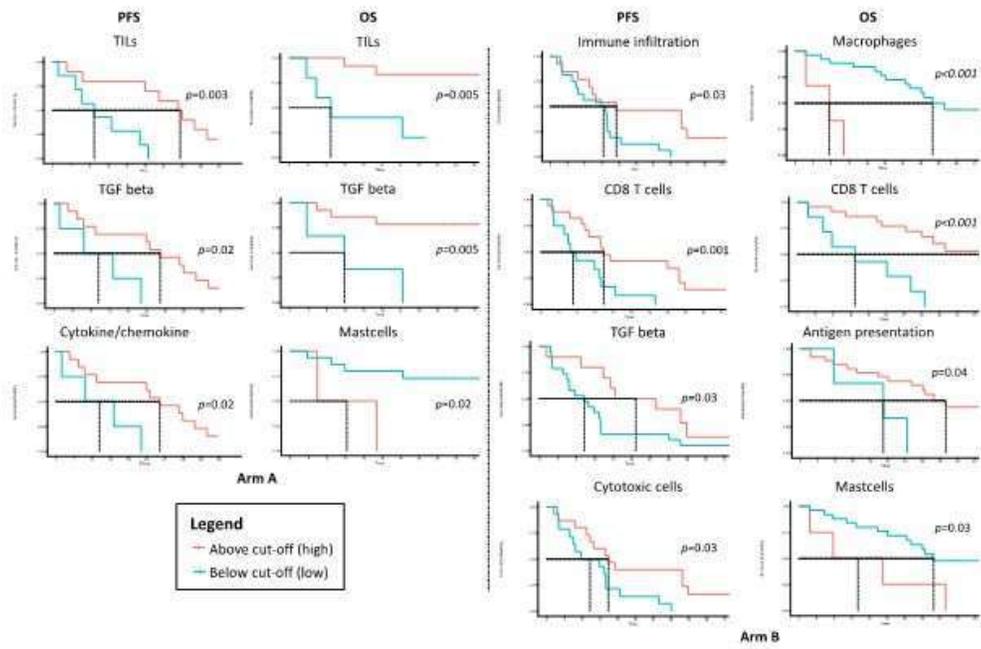
PFS — **OS** — **PFS** — **OS**

TILs — TILs — Immune infiltration — Macrophages

p=0.003 — p=0.005 — p=0.03 — p<0.001

TGF beta — TGF beta — CD8 T cells — CD8 T cells

p=0.02 — p=0.005 — p=0.001 — p<0.001

Cytokine/chemokine — Mastcells — TGF beta — Antigen presentation

p=0.02 — p=0.02 — p=0.03 — p=0.04

Arm A

Cytotoxic cells — Mastcells

p=0.03 — p=0.03

Legend
— Above cut-off (high)
— Below cut-off (low)

Arm B

Regarding TLS and immune pattern, albeit not statistically significant, some trends were observed (Figures 6-7).

Figure 6. Survival trends according to TLS presence and treatment arm

A

No TLS   TLS w/o GC   TLS with GC

B

Arm A

Arm B
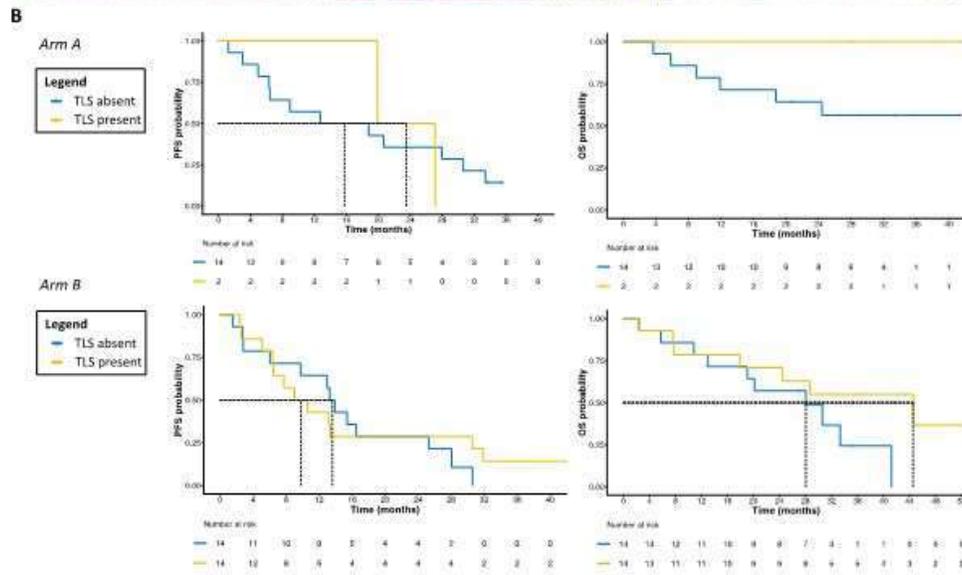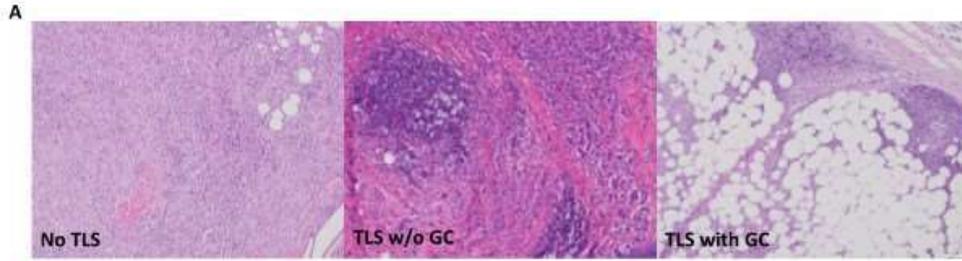
Legend. A: Representative images of TILs infiltration without TLS, with TLS and GC or without GC, at hematoxylin and eosin staining. All pathology images in these panels are magnified at 10X. B: Exploratory Kaplan-Meier curves of PFS and OS according to study population, based on presence/absence of TLS. The "TLS present" category included TLS with/without clearly visible GC. TILs: tumor-infiltrating lymphocytes; TLS: tertiary lymphoid

structures, including every lymphoid aggregates more or less organized; w/o: without; GC: germinal center; PFS: progression-free survival; OS: overall survival.

Figure 7. Survival trends according to immune pattern and treatment arm

A

Desert   Excluded   Inflamed

B

Arm A

Arm B

Legend. A: Representative images of different immune infiltration patterns at hematoxylin and eosin staining. All pathology images in these panels are magnified at 10X. B: Exploratory Kaplan-Meier curves of PFS and OS according to study population, based on immune infiltration pattern.

Namely, in arm A the immune desert pattern, compared to the immune inflamed showed lower PFS (median 15.8 [95% CI 6.4-NE] vs. 27.5 [95% CI 27.1-NE]) and OS (mOS NE in both cases), as well as the absence of TLS, compared to the presence of TLS, with or without germinal centers (mPFS 15.8 [95% CI 6.4-NE] vs. 23.5 [95% CI 19.9-NE] months; mOS NE in both cases). In arm B there was no clear association in terms of PFS, but patients with immune inflamed tumors, compared to immune desert and excluded, did not reach mOS at the time of analysis (NE [95% CI 7.6-NE] vs. 28.7 [95% CI 19.1-NE] vs. 28.1 [95% CI 28.06-NE], respectively), and the presence of TLS was associated to longer OS (44.5 [95% CI 24.5-NE] vs. 28.1 [95% CI 19.0-NE]).

A trend for association with ORR was observed for the immune inflamed pattern and TLS presence in arm A (OR>1.0 in both cases), not in arm B.

To better explore the composition of TILs in our cohort and their association with tumor biology, we carried out multiple Pearson's correlations with immune signatures and some immune genes of interest or associated to specific immune cell types, as well as with PAM50 IS scores and ROR-P. TILs were weakly positively correlated to the HER2E score, antigen presentation, macrophages,

CD8 T cells, immune infiltration and cytotoxic cells signatures and *LAG3* (representing exhausted T cells) levels and moderately positively correlated to *CD19* levels (**Figure 8**).

Figure 8. Correlation matrix of associations among TILs and immune genes/signatures and ROC curves for the predictive capacity of *CD19* and *CXCL13*

A

B

CD19 mRNA

CXCL13 mRNA
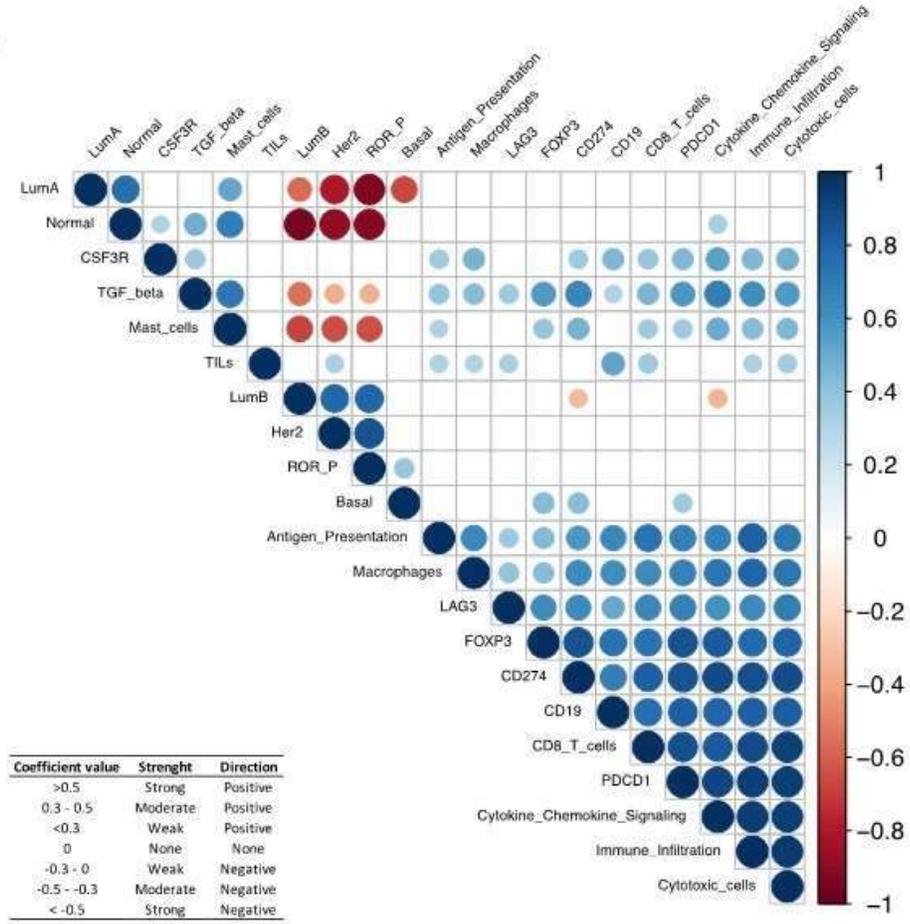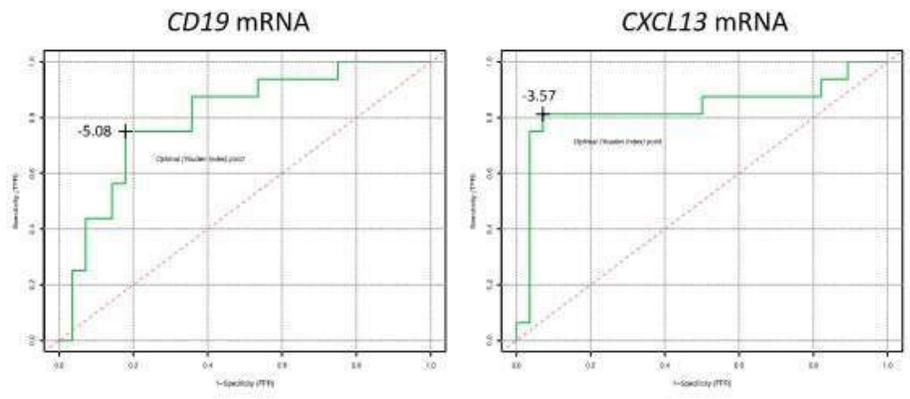
Legend. A: correlation matrix; B: ROC curves assessing the capacity of CD19's and CXCL13's relative transcript abundance to predict the presence of tertiary lymphoid structures. FPR: false positive rate; TPR: true positive rate. In panel A, only significant correlations for $p<0.05$ are represented as colored dots. The darker blue the more positive the association, while the darker orange, the more neg ative the correlation according to the Pearson coefficient value. In panel B, mRNA values selected according to Youden Index are reported.

Finally, TILs (OR=1.40, 95% CI 1.12–1.75, p=0.004), *CD19* (OR=1.81, 95% CI 1.17–2.81, p=0.008) and *CXCL13* (OR=2.57, 95% CI 1.47–4.51, p=0.001) mRNA levels were significantly associated to the presence of TLS, differently from PAM50 signatures, other immune genes/signatures and ROR-P (all p>0.05). *CD19* and *CXCL13* predicted TLS presence with an AUC of 0.80 (95% CI 0.66-0.95) and 0.83 (95% CI 0.70-0.97), respectively, demonstrating very good predictive capacity. Exploratory cut-offs obtained with Youden Index are reported in Figure 8.

e) Statistical Issues

All statistical analyses were carried out according to planned analysis reported in the protocol; for Progression-Free Survival analysis, all dropout or withdrew were censored at the time of last visit, no adjustments for unbalances or for multiple comparisons were made; Hazard Ratio for Progression-Free Survival estimation was unadjusted. No interim analysis was performed.

f) Drug Dose, Drug Concentration and Relationship to Response

Since no patients reduced or delayed the treatment,, the analysis of the relation between drug dose and response was not applicable.

g) Drug-drug and drug-disease interactions

The efficacy variables are not influenced by the previous or concomitant treatment.

h) Efficacy Conclusions

In summary, as regards the population eligibility criteria, the type of treatment regimen, the timing of the disease assessment highlights that the KENDO trial, though closed prematurely, adds further evidence supporting CDK4/6i+ET use in aggressive HR+/HER2-neg. MBC instead of chemotherapy. PAM50 IS, genomic and immunological features are promising biomarkers to personalise therapeutic choices.

10.3   Safety Evaluation

a) Extent of Exposure

All 49 patients included in the efficacy analysis are included in the safety analysis. The duration of treatment and the dose of the study drugs are already reported by each treatment arm in Tables 2 and 4.

b) Adverse Events

The overall incidence of adverse events, classified by NCI CTCAE v5.0 toxicity grading, are reported in Table 3.

c) Deaths

Four deaths were observed during the study directly associated to tumor progression, 1 in arm A and 3 in arm B. Cross-over upon PD concerned 4 patients in total, 3 from the CT arm and 1 from the CDK4/6-inhibitors arm.

d) Other Serious

None.

e) Adverse Events Leading to Withdrawal

None.

f) Other Significant Adverse Events

None

g) Narratives

This study is not to be included in a regulatory dossier.

h) Clinical Laboratory Evaluations

All clinically significant changes are reported in the adverse event section.

i) Vital signs, Physical Findings and Other Observations Related to Safety

All clinically significant changes are reported in the adverse event section.

j) Safety Conclusion

We didn't observe any clinically relevant and unexpected adverse event. The most common (≥5%) grade 3–4 AEs in both arms was neutropenia (41.2% in arm A and 22% in arm B, respectively). A detailed report of AEs can be found in Table 3. No patient stopped study treatment because of toxicity. Among patients treated in arm A, 2 of them (12%) required a dose reduction, instead in the arm B, 10 (31%) patients. No serious adverse events have been reported.

11 Discussion and Overall Conclusions

Our study is an example of Comparative Effectiveness Research (CER) promoted to reduce the gap between clinical research, in particular that promoted by pharmaceutical companies and clinical practice, and to highlight the role of non-profit clinical trials supported by public institution.

The KENDO randomized phase II trial was designed to demonstrate the superiority of chemotherapy (CT), +/- endocrine therapy (ET), with respect to CDK4/6-inhibitors+ET in patients with HR+/HER2-negative metastatic breast cancer (MBC) exhibiting aggressive characteristics or endocrine resistance. While the trial closed prematurely and failed to meet its primary endpoint, intriguing trends emerged from tissue biomarker analyses. While PAM50 Luminal A tumors showed poor outcomes when treated with CT, Basal-like tumors responded poorly to CDK4/6-inhibitors+ET. Moreover, research-based PAM50 ROR-P categories predicted response to CDK4/6-inhibitors-based regimens. Interestingly, high levels of genes/ signatures of pathways involved in tumor survival and proliferation were associated with worse outcomes, compared to immune-related genes/signatures, especially in the CDK4/6-inhibitors arm. Notably, tertiary lymphoid structures and higher tumor-infiltrating lymphocytes also showed favorable survival trends with CDK4/6-inhibitors+ET. These findings highlight the importance of considering tumor biology and immune composition for optimizing therapeutic strategies in HR+/HER2-negative MBC. Confirmatory studies are required.

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Appendix 1

List of Principal Investigators and Centers

Principal Investigator	City	Department	Center
~~Andrea Rocca~~ Ugo De Giorgi	Meldola-Forlì-Cesena	U.O. Oncologia Medica	IRST IRCCS *Coordinating Center*
~~Laura Amaducci~~ Claudio Dazzi	Ravenna-Lugo-Faenza	U.O. Oncologia Medica	Ospedale di Ravenna
Lorenzo Gianni	Rimini-Cattolica	Oncologia ed Oncoematologia	Ospedale degli Infermi
Fabrizio Artioli	Carpi	Dip. Medicina Interna e Riabilitazione – U.O. Medicina Interna Oncologica	Ospedale "Ramazzini"
Alba Brandes	Bologna	U.O. Oncologia Medica	P.O. Bellaria-Maggiore
Luigi Cavanna	Piacenza	Dip. Oncologia-Ematologia – U.O. Oncologia Medica	Azienda USL di Piacenza - Ospedale "Guglielmo da Saliceto"
Vito Lorusso	Bari	U.O. Oncologia Medica	Ist. Tumori Giovanni Paolo II - IRCCS Osp. Oncologico di Bari
Antonio Musolino	Parma	U.O. Oncologia Medica	AOU di Parma
Daniele Generali	Cremona	Terapia Molecolare e Farmaco Genomica	Azienda Socio-Sanitaria Territoriale di Cremona
Filippo Giovanardi	Guastalla	U.O. Oncologia Medica	Ospedale Civile di Guastalla - AUSL di Reggio Emilia
Giancarlo Bisagni	Reggio Emilia	U.O. Oncologia Medica	A.O. Arcispedale S. Maria Nuova IRCCS di Reggio Emilia
Federico Piacentini	Modena	DH Oncologico	A.O.U. Policlinico di Modena
Alessandro Bertolini	Sondrio	U.O.C. Oncologia Medica	Ospedale di Sondrio - ASST Valtellina e Alto Lario
Fausto Roila	Perugia	S.C. Oncologia Medica	A.O. Santa Maria della Misericordia di Perugia
Antonio Maestri	Imola	U.O.C Oncologia Medica	AUSL Imola
Rossana Berardi	Ancona	Clinica Oncologica	A.O.U. Ospedali Riuniti Umberto I - GM Lancisi - G Salesi
Alessandra Gennari	Novara	S.C. Oncologia	A.O.U. Maggiore della Carità di Novara
Nicola Battelli	Macerata	U.O.C. Oncologia	Ospedale di Macerata, ASUR AV3
Andrea Bonetti	Legnago	U.O. Oncologia Medica	Ospedale Mater Salutis – Azienda ULSS9 Scaligera
Antonio Frassoldati	Ferrara	Oncologia Clinica	A.O.U. di Ferrara Arcispedale Sant'Anna

Appendix 2

Independent Ethical Committee	Participating center	Approval of protocol First Version - 03.03.2017	Approval of protocol Amendment 1.0 - 09.11.2018
C.E.ROM. (Comitato Etico della Romagna)	IRCCS IRST (Coordinating center)	12.07.2017	12.12.2018
	Ravenna, Lugo, Faenza	12.07.2017	12.12.2018
	Rimini, Cattolica	12.07.2017	12.12.2018
AVEN (Comitato Etico Area Vasta Emilia Nord)	Modena	NA	10.09.2019
	Guastalla	NA	02.04.2019
	Reggio Emilia	NA	02.04.2019
	Carpi	25.07.2017	05.02.2019
	Parma	09.11.2017	05.02.2019
	Piacenza	19.09.2017	05.02.2019
AVEC (Comitato Etico Area Vasta Emilia Centrale)	Bologna	25.05.2017	20.03.2019
	Imola	NA	20.03.2019
	Ferrara	29.09.2017	20.03.2019
Comitato Etico Ist. Tumori Giovanni Paolo II - IRCCS Osp. Oncologico di Bari	Bari	21.09.2017	NA
Comitato Etico Val Padana	Cremona	29.09.2017	18.02.2019
Comitato Etico per la Sperimentazione Clinica delle Provincie di Verona e Rovigo	Legnago	NA	09.01.2019
Comitato Etico Regionale delle Marche	Macerata	NA	09.05.2019
	Ancona	NA	28.03.2019
Comitato Etico Brianza	Sondrio	NA	07.02.2019
Comitato Etico Regionale dell'Umbria	Perugia	NA	22.01.2019
Comitato Etico Interaziendale AOU "Maggiore della Carità" di Novara	Novara	NA	10.05.2019

IRST174.19 KENDO
List of Ethics Committees

FOGLIO INFORMATIVO PER IL PAZIENTE

Sigla di identificazione dello studio: IRST174.19

Titolo dello studio: Studio clinico randomizzato di confronto tra chemio-endocrinoterapia concomitanti e chemioterapia seguita da endocrinoterapia come trattamento di prima linea del carcinoma mammario metastatico luminale B.

Data e Versione N°: 03/03/2017 – Versione Finale

Sperimentatore Principale: _____

Promotore dello studio: Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS
Via Piero Maroncelli, 40/42
47014 Meldola (FC)

Nome e Cognome del paziente: _____

Gentile Sig.ra/Sig.re, le informazioni contenute nella scheda seguente intendono essere esaurienti e sono quindi DETTAGLIATE e COMPLESSE. Le chiediamo di accettare la partecipazione allo studio SOLO dopo avere letto con attenzione la scheda ed avere avuto un colloquio ESAURIENTE con il medico sperimentatore che le dovrà dedicare il TEMPO NECESSARIO per una comprensione di ciò che le viene proposto.

Introduzione

Gent. Sig.ra/re, Le viene chiesto di partecipare ad una ricerca clinica dal titolo "*Studio clinico randomizzato di confronto tra chemio-endocrinoterapia concomitanti e chemioterapia seguita da endocrinoterapia come trattamento di prima linea del carcinoma mammario metastatico luminale B*".

Prima di decidere se partecipare o meno, è importante che Lei venga informato/a dei motivi per i quali la ricerca viene condotta e cosa comporterà per Lei la partecipazione alla ricerca. Si prenda tutto il tempo necessario per leggere queste informazioni attentamente e, se lo desidera, ne discuta con amici, parenti e col Suo medico di famiglia.
Non esiti a fare domande se qualcosa (ad esempio qualche termine medico o espressione) non Le è chiaro o se desidera avere maggiori informazioni.

Chi sta conducendo questa ricerca e quanti pazienti saranno coinvolti?

Questa ricerca coinvolgerà circa 300 pazienti in Italia eviene condotta presso l'Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS (promotore e centro coordinatore),

sotto la responsabilità del dott. Andrea Rocca e dal Centro di
_____, sotto la responsabilità di _____.

Perché Le è stato chiesto di partecipare a questo studio?
Il Medico che la cura Le ha proposto di partecipare a questa ricerca in quanto Lei risulta affetta/o
da carcinoma (un tipo di tumore) della mammmella localmente avanzato (ossia diffuso dalla
mammella ai tessuti adiacenti quali la cute, i linfonodi, o le strutture della parete toracica) non
suscettibile di trattamento chirurgico o radioterapico radicali (ossia in grado di eradicare
completamente la malattia), oppure metastatico (ossia diffuso ad organi distanti dalla mammella,
quali scheletro, fegato, polmoni, o altri), quindi non trattabile con chirurgia o radioterapia, ma
bensì principalmente mediante l'uso di farmaci. In particolare si tratta di un carcinoma cosiddetto
"luminale", che è caratterizzato dalla presenza di "recettori per gli estrogeni" (o recettori
estrogenici), molecole a cui si legano gli ormoni estrogeni per svolgere le loro funzioni nelle cellule,
funzioni che nel caso delle cellule mammarie comprendono lo stimolo alla proliferazione cellulare.
In sostanza gli estrogeni, legandosi ai recettori estrogenici, stimolano la crescita dei carcinomi
mammari luminali. Pertanto una modalità efficace di trattamento dei carcinomi mammari luminali
consiste nell'impiego di farmaci che bloccano la produzione di estrogeni nell'organismo.
Genericamente, le terapie che agiscono sugli ormoni vengono indicate con il termine di "terapie
ormonali", o con quello equivalente di "terapie endocrine" o "endocrinoterapia".
Nelle donne in pre-menopausa gli estrogeni vengono prodotti principalmente dalle ovaie e la loro
produzione può essere bloccata mediante farmaci chiamati "agonisti dell'ormone di rilascio delle
gonadotropine" (GnRH agonisti), detti anche "agonisti dell'ormone di rilascio dell'ormone
luteinizzante" (LHRH agonisti). Questi farmaci bloccano il ciclo mestruale e la produzione di
estrogeni da parte delle ovaie.
Nelle donne in post-menopausa (e in quelle in pre-menopausa in trattamento con LHRH agonisti)
la produzione di estrogeni avviene da parte di altri tessuti, in primo luogo il tessuto adiposo, e può
essere bloccata mediante farmaci chiamati "inibitori dell'aromatasi" (aromatasi è il nome
dell'enzima che favorisce la produzione degli estrogeni).
Pertanto, una adeguata soppressione della produzione di estrogeni si ottiene mediante un
inibitore dell'aromatasi nelle donne in post-menopausa e mediante la combinazione di LHRH
agonista + inibitore dell'aromatasi nelle donne in pre-menopausa. L'inibitore dell'aromatasi è
inoltre efficace come terapia del carcinoma mammario in soggetti maschi, nei quali viene utilizzato
da solo o in combinazione con LHRH agonista.
Attualmente la malattia da cui Lei è affetta/o viene trattata con chemioterapia nei casi, come il
Suo, in cui vi è stata una ricaduta della malattia a breve distanza di tempo dall'intervento
chirurgico, o una precoce progressione della malattia in corso di una precedente terapia ormonale
effettuata per malattia avanzata, o la presenza di lesioni che causino sintomi/disturbi importanti, o
un interessamento esteso di organi vitali o un iniziale malfunzionamento degli stessi, oppure in
presenza di alcune caratteristiche tumorali quali un basso contenuto di recettori ormonali o una
elevata quota di cellule proliferanti. In tutti questi casi infatti le probabilità di rispondere a una
chemioterapia sono maggiori rispetto alle probabilità di rispondere a una terapia ormonale,

oppure la chemioterapia può favorire un più rapido controllo della malattia, ragione per cui si preferisce il trattamento chemioterapico. Se si ottiene una buona risposta alla chemioterapia, si usa poi passare ad una terapia ormonale di mantenimento.

Esistono tuttavia studi di laboratorio (e anche alcuni studi clinici su pazienti) che mostrano come l'utilizzo concomitante, simultaneo, di chemioterapia e terapia endocrina possa risultare più efficace rispetto alla somministrazione della chemioterapia da sola.

Questo studio si propone pertanto di confrontare il trattamento concomitante chemio-endocrino, in cui chemioterapia e terapia ormonale vengono iniziati e proseguiti contemporaneamente (e che chiameremo di seguito "trattamento concomitante"), con il trattamento standard che è rappresentato da una chemioterapia somministrata da sola per un certo periodo, a cui si fa seguire una endocrinoterapia, anch'essa somministrata da sola per un dato periodo (chiameremo di seguito questa modalità di cura "trattamento sequenziale"). Per effettuare questo confronto, le/i pazienti vengono suddivisi in due gruppi, un gruppo che riceve il trattamento concomitante e uno che riceve quello sequenziale.

Per poter fare un confronto obbiettivo tra i due trattamenti è necessario che i pazienti messi in ciascuno dei due gruppi non siano selezionati in base a caratteristiche che possano influenzare l'esito del trattamento (ad esempio, mettere tutti i pazienti con malattia più aggressiva in un gruppo e tutti quelli con malattia più blanda nell'altro favorirebbe nei risultati il trattamento attribuito al secondo gruppo, in maniera artificiosa, provocando quella che in termini tecnici viene definita una "distorsione" nei risultati dello studio). Pertanto si rende necessario utilizzare, nell'attribuzione dei pazienti ai due gruppi di trattamento, il processo di randomizzazione, che consiste nell'attribuire i pazienti ai gruppi in maniera casuale: per ogni paziente incluso nello studio viene "estratto a sorte" il gruppo in cui quel paziente verrà inserito. Il processo di randomizzazione è svolto tramite un programma di computer, ma è sostanzialmente equivalente a lanciare una moneta ed attribuire il paziente ad un gruppo se esce testa e all'altro gruppo se esce croce.

Il fatto che il gruppo di trattamento venga scelto "a caso" non dovrebbe essere motivo di preoccupazione, dal momento che non è ad oggi noto quale sia il trattamento migliore per i pazienti (se quello concomitante o quello sequenziale): tradizionalmente nella pratica clinica si usa il trattamento sequenziale ma alcuni studi biologici indicano che quello concomitante potrebbe essere migliore. Perciò, nel caso in cui la randomizzazione porti all'attribuzione del trattamento sequenziale, che è quello oggi considerato standard, si riceve il trattamento che si riceverebbe anche al di fuori dello studio; nel caso in cui la randomizzazione porti all'attribuzione del trattamento concomitante, si riceve un trattamento che potrebbe teoricamente essere migliore (sebbene ciò rimanga da dimostrare nei pazienti e saranno questo studio e studi analoghi in corso a confermare o smentire questa ipotesi) e che non è però ancora utilizzabile nella pratica clinica, non essendo ancora supportato dai risultati di studi clinici.

Qual è il farmaco che viene sperimentato in questa ricerca?
La scelta del tipo di chemioterapia è a discrezione del Suo oncologo, il quale sceglierà il o i chemioterapici che ritiene più indicati in base alle caratteristiche della Sua malattia e alle Sue

condizioni generali. Si tratta comunque e sempre di farmaci già di uso corrente, dei quali sono ben noti efficacia e tossicità.

La scelta del trattamento ormonale è invece ristretta all'uso degli inibitori dell'aromatasi (a cui andrà associato un LHRH agonista nelle donne in pre-menopausa ed eventualmente negli uomini). Esistono 3 farmaci appartenenti alla classe degli inibitori dell'aromatasi: anastrozolo, letrozolo, ed exemestane; i primi due sono di tipo "non steroideo" e l'ultimo di tipo "steroideo" (questi termini si riferiscono alla struttura chimica di tali farmaci). L'unico vincolo nella scelta di quale inibitore dell'aromatasi assumere nello studio è che andrà utilizzato un inibitore di tipo steroideo se vi è già stata una progressione tumorale in corso di precedente terapia con un inibitore non steroideo e viceversa. Anche questi sono farmaci già di uso corrente e di cui sono noti efficacia e tossicità.

Ciò che è nuovo in questa ricerca non sono pertanto i farmaci utilizzati, bensì il fatto che chemioterapici e farmaci ormonali vengano utilizzati contemporaneamente e non in sequenza gli uni dopo gli altri come avviene secondo pratica clinica.

Qual è lo scopo della ricerca?

Lo scopo di questa ricerca è confrontare l'efficacia del trattamento chemio-endocrino concomitante con quella del trattamento standard sequenziale con chemioterapia seguita da endocrinoterapia. L'efficacia in questo studio viene valutata in termini di capacità del trattamento di tenere sotto controllo la malattia tumorale, ossia di evitare un peggioramento della neoplasia (in termini tecnici il peggioramento è indicato come "progressione" di malattia). Ripetendo una TAC circa ogni 3 mesi dall'inizio del trattamento, si valuta ogni volta se la malattia sia migliorata (si parla allora di "risposta" di malattia, che può essere parziale o completa), o stabile/invariata (si parla di "stabilità" di malattia), o peggiorata ("progressione"). Sia la risposta che la stabilità di malattia stanno a indicare che il trattamento sta efficacemente contrastando la malattia, mentre la progressione di malattia indica che il trattamento non è efficace o ha esaurito la sua efficacia. Pertanto, per ogni paziente si misurerà il tempo intercorso tra l'inizio del trattamento e il riscontro di una progressione di malattia, e questo rappresenterà il tempo durante il quale la malattia è rimasta controllata dal trattamento (in termine tecnico definito come "intervallo libero da progressione di malattia"). Calcolando una sorta di tempo medio libero da progressione di malattia per ognuno dei due gruppi di trattamento e confrontanto tale tempo medio tra i due gruppi, si vedrà quale trattamento risulterà più efficace.

Come detto sopra, in base alle conoscenze già disponibili si ritiene che il trattamento concomitante possa risultare più efficace: lo studio che Le è stato proposto è stato disegnato per dimostrare con metodo scientifico la validità di questa ipotesi.

Per fare questo, dopo aver valutato l'estensione della malattia nell'organismo (mediante esami diagnostici come TAC e/o altri come risonanza magnetica, ecografia, scintigrafia ossea, PET) e aver controllato la funzionalità degli organi principali (midollo oseeo, fegato, reni) con esami ematici, e dopo aver verificato che siano rispettati tutti i criteri di inclusione nello studio, avverrà la randomizzazione, ossia l'attribuzione casuale a uno dei due gruppi di trattamento: concomitante o sequenziale. Iniziato il trattamento, la valutazione di efficacia avverrà, come detto sopra, mediante la ripetizione degli esami diagnostici (TAC e/o altri) circa ogni 3 mesi e la prosecuzione o meno del

trattamento dipenderà dall'esito di tali esami. In caso di riscontro di "progressione" di malattia il trattamento verrà sospeso, mentre in caso di stabilità o risposta il trattamento verrà proseguito. In ogni caso la somministrazione della chemioterapia avverrà (a meno di effetti tossici che la rendano intollerabile) per un minimo di 3 mesi e di solito fino a 6-9 mesi (anche di più in caso di chemioterapie molto ben tollerate), dopo di che, una volta raggiunto il massimo beneficio (in termini di riduzione delle masse tumorali) ottenibile con quel trattamento, si potrà proseguire con la sola endocrinoterapia (ciò sia nel gruppo trattato con terapia concomitante, nel quale verrà sospesa la chemioterapia e proseguita la sola endocrinoterapia, sia nel gruppo trattato con terapia sequenziale, nel quale verrà sospesa la chemioterapia e iniziata la terapia endocrina). Nel gruppo sottoposto a terapia sequenziale, la prosecuzione con terapia endocrina potrà avvenire anche in caso di progressione alla chemioterapia, nei casi in cui il medico lo ritenga clinicamente indicato (e non reputi invece necessario passare ad un'altra chemioterapia con farmaci diversi da quelli utilizzati in precedenza).

La durata complessiva del trattamento non è definibile a priori ed il trattamento proseguirà fino ad esaurimento dell'efficacia (progressione di malattia) o al verificarsi di effetti tossici non tollerabili. Nel gruppo che riceve la terapia sequenziale, se dopo progressione alla chemioterapia il medico riterrà indicato procedere comunque con la terapia endocrina, si considererà definitivamente esaurita l'efficacia del trattamento al momento della progressione a quest'ultima.

Esistono dei trattamenti alternativi?

Se Lei non volesse partecipare alla ricerca che Le viene proposta, verrebbe curato/a con un trattamento chemioterapico, eventualmente seguito da un trattamento ormonale di mantenimento.

Se Lei deciderà di non partecipare a questa ricerca, il Medico Responsabile della ricerca valuterà con Lei il rapporto rischio/beneficio di tutte le possibili strategie terapeutiche attualmente disponibili per la cura della Sua malattia. Si senta libera/o di porre tutte le domande che desidera sulle diverse opzioni di trattamento.

A cosa andrà incontro se accetterà di partecipare alla ricerca?

Qualora Lei accetti di partecipare a questa ricerca, dovrà firmare il modulo di Consenso Informato e il Medico Responsabile della ricerca Le chiederà di sottoporsi ai seguenti esami/procedure:

- raccolta della storia clinica, inclusi i farmaci assunti per altre patologie*
- visita medica*
- registrazione parametri vitali (pressione, frequenza cardiaca, temperatura, oltre a peso e statura)*
- esami di laboratorio (sangue ed eventualmente urine)*
- TAC torace e addome completo con mezzo di contrasto (in caso di allergia al mezzo di contrasto TAC, si possono effettuare TAC torace senza mezzo di contrasto e Risonanza Magnetica dell'addome, possibilmente con mezzo di contrasto)
- eventuali altri esami diagnostici quando indicato, in base ai sintomi presenti o ad alterazione di esami di sangue (es. scintigrafia ossea, TAC o Risonanza Magnetica di altri settori)

- eventuale valutazione cardiologica con elettrocardiogramma, visita e/o ecocardiogramma se clinicamente indicato (in base alle patologie associate e al tipo di chemioterapia scelta)*
- valutazione della qualità di vita con appositi questionari: EORTC QLQ-C30 e QLQ-BR23.

Di questi esami, quelli indicati con l'asterisco farebbero comunque parte delle indagini necessarie per diagnosticare e trattare la patologia da cui è affetto/a. Inoltre, la TAC con mezzo di contrasto è anch'essa frequentemente utilizzata nella pratica clinica (al di fuori di studi clinici) sebbene possa talvota essere efficacemente sostituita da radiografia del torace ed ecografia dell'addome.

Se Lei è una donna in età potenzialmente fertile, verrà effettuato anche un test di gravidanza da un campione di sangue o di urine, al fine di essere certi che Lei non sia incinta per non correre il rischio di esporre il nascituro agli effetti potenzialmente tossici del trattamento sperimentale.

Gli esami elencati sopra verranno ripetuti periodicamente nel corso dello studio: alcuni esami ematici prima di ogni ciclo di chemioterapia, gli esami per valutare l'estensione della malattia tumorale (TAC e/o altri visti sopra) circa ogni 3 mesi, gli accertamenti cardiologici in base alla situazione clinica.

Lo studio prevede anche la raccolta e la valutazione di campioni biologici di sangue e tessuto tumorale destinati ad indagini di biologia molecolare. Tali indagini hanno lo scopo di identificare dei fattori biologici (proteine, o mutazioni di geni) che aiutino a predire quali pazienti beneficeranno maggiormente del trattamento concomitante piuttosto che di quello sequenziale. I risultati di questa ricerca potranno essere utili in futuro per il trattamento di pazienti affetti dalla Sua stessa patologia.

Si tratta in particolare di:

- utilizzo di un campione di tessuto tumorale derivato dal Suo tumore primitivo e conservato presso l'Anatomia Patologica del centro in cui ha eseguito la biopsia del tumore o l'intervento chirurgico di asportazione del tumore mammario
- un prelievo di sangue (10 ml) eseguito prima di iniziare il trattamento e ripetuto circa ogni 3 mesi per la durata dello studio
- un ulteriore prelievo di sangue (10 ml) eseguito prima di iniziare il trattamento, ripetuto dopo circa 2 mesi di trattamento e al termine della chemioterapia.

Se Lei accetterà di aderire, accetterà di fornire un campione di tessuto tumorale derivato dal Suo tumore già archiviato presso l'Anatomia Patologica del Centro, e di sottoporsi ai prelievi di sangue periodici sopracitati. La parte biologica è infatti integrante allo studio clinico ed obbligatoria per ciascun paziente che partecipa alla sperimentazione.

Farmaci dello studio - Trattamenti

Se il Medico responsabile della ricerca La riterrà idonea/o a partecipare alla ricerca, Lei riceverà i seguenti trattamenti:

- <u>un trattamento chemioterapico</u>, composto da uno o più farmaci chemioterapici, la cui scelta è a discrezione del suo oncologo e si baserà sulle caratteristiche della Sua malattia e sulle Sue condizioni generali. Si tratta comunque e sempre di farmaci già di uso corrente, dei quali sono ben noti efficacia e tossicità. Alcuni chemioterapici vengono somministrati per via endovenosa (e possono richiedere l'impianto di un catetere venoso), altri per bocca.

La somministrazione avviene più spesso a "cicli", soprattutto per i farmaci utilizzati per via endovenosa, con somministrazioni ad esempio una volta ogni 3 settimane; altre volte le somministrazioni si fanno una volta alla settimana o talora, di solito per farmaci orali, tutti i giorni. Il tipo e l'entità degli effetti collaterali variano in base agli specifici farmaci utilizzati, ma in generale i principali effetti collaterali possibili sono i seguenti:
-   stanchezza
-   nausea e vomito
-   sapore cattivo o alterazione del gusto
-   perdita di appetito
-   infiammazione della bocca, con dolore e possibili ulcere
-   infiammazione della gola e dell'esofago, con dolore alla deglutizione
-   disturbi intestinali come diarrea o stitichezza
-   infiammazione delle congiuntive
-   infiammazione della vescica con bruciore ad urinare
-   diminuzione del numero di globuli bianchi, le cellule del sangue che difendono dalle infezioni, quindi maggiore suscettibilità alle infezioni (febbre, talvolta con brividi, e disturbi legati alla sede dell'infezione, come tosse, mal di gola, bruciore a urinare, ecc)
-   diminuzione del numero di globuli rossi (anemia) che provoca stanchezza e talvolta difficoltà di respiro
-   diminuzione del numero di piastrine, le cellule del sangue che proteggono da sanguinamenti, con possibili sanguinamenti dalle mucose (es. dal naso, epistassi) o a livello della pelle con formazione di piccole macchie rossastre (petecchie) o di lividi (ecchimosi)
-   caduta di capelli e peli e talvolta di ciglia e sopracciglia
-   alterazioni della pelle
-   disturbi alle unghie che possono diventare secche, scheggiate o striate
-   neuropatia periferica, un disturbo dei nervi periferici che si manifesta con alterazioni della sensibilità, formicolii, sensazione di punture di spilli soprattutto alle mani e ai piedi (in genere regredisce al termine delle cure, ma solo dopo diversi mesi)
-   riduzione dell'udito
-   alterazione della funzionalità ovarica con blocco transitorio o permanente dei cicli mestruali nella donna, possibile sterilità nell'uomo.
Inoltre, assai raramente, possono verificarsi alcune tossicità più severe, come:
-   riduzione della funzionalità del cuore
-   alterazioni della funzionalità del fegato
-   alterazioni della funzionalità dei reni
-   polmoniti di tipo non infettivo
-   formazione di trombi, ossia coaguli di sangue all'interno di una vena o un'arteria.

La maggior parte dei disturbi elencati regredisce una volta completato il trattamento; tuttavia alcuni farmaci non possono essere somministrati oltre un certo dosaggio complessivo per non incorrere nel rischio di riduzioni irreversibili della funzionalità di alcuni organi. L'oncologo che l'ha in cura le spiegherà in dettaglio i possibili effetti collaterali del trattamento prescelto e attuerà tutte le misure atte a contenere tali effetti e ad evitare problemi a lungo termine.

- un trattamento ormonale, consistente, come visto sopra, in un farmaco della categoria degli inibitori dell'aromatasi (anastrozolo, letrozolo, o exemestane), a cui andrà associato un LHRH agonista nelle donne in pre-menopausa e negli uomini. I principali possibili effetti collaterali degli inibitori dell'aromatasi, da soli o associati a LHRH analoghi, sono i seguenti:
    - vampate di calore e sudorazione abbondante
    - dolori articolari e osteo-muscolari
    - riduzione della densità ossea (osteopenia / osteoporosi)
    - aumento di colesterolo e trigliceridi nel sangue
    - sbalzi di umore
    - senso di stanchezza e affaticamento
    - difficoltà di memoria
    - aumento di peso
    - secchezza vaginale
    - calo del desiderio sessuale
    - capogiri
    - cefalea
    - cataratta, irritazioni agli occhi, visione offuscata
    - aumento degli enzimi epatici
    - caduta capelli
    - alterazioni della pelle
    - prurito
    Inoltre, con le terapie ormonali si è riscontrato:
    - un lieve aumento del rischio di disturbi della circolazione venosa o arteriosa (tromboflebite, embolia polmonare, trombosi arteriosa, infarto miocardico o cerebrale), eventi che rimangono comunque molto rari.

L'assegnazione ai bracci di trattamento (terapia concomitante o sequenziale) verrà operata in modo randomizzato, cioè determinato dal caso. La randomizzazione, infatti, consiste nell'attribuzione rigorosamente casuale, mediante un apposito sistema informatico, di ogni paziente che man mano viene ammesso a uno dei bracci di trattamento.

Modalità di Assunzione dei farmaci
La somministrazione di molti chemioterapici avviene per via endovenosa ed è effettuata in ospedale. L'oncologo che la segue le spiegherà le modalità di somministrazione dei vari farmaci,

nonché eventuali terapie da assumere a domicilio per contrastare gli effetti collaterali dei chemioterapici. Alcuni dei chemioterapici vanno assunti per bocca e sarà il suo oncologo a spiegarle esattamente quando, in quali dosi e come assumere questi farmaci a domicilio e a prescrivere eventuali altri farmaci da assumere per contrastare gli effetti collaterali dei chemioterapici. Le verrà spiegato inoltre come conservare i farmaci a domicilio. **Tutti i farmaci, chemioterapici e non, devono essere conservati lontano dalla portata dei bambini**.

Quanto durerà lo studio?
Per l'esecuzione delle visite e degli accertamenti previsti dallo studio dovrà recarsi in Ospedale un numero di volte variabile in base al tipo di chemioterapia utilizzato (in genere ogni 3-4 settimane, per alcuni schemi di chemioterapia una volta alla settimana), per tutto il periodo di trattamento previsto dallo studio (ossia fino a interruzione del trattamento per progressione di malattia, tossicità, o per sua decisione o per decisione del suo oncologo).
Il Medico responsabile della ricerca potrà decidere di interrompere il trattamento in studio nel caso in cui si manifestino dei problemi importanti per la Sua salute che non Le consentano di continuare la partecipazione allo studio.
Talvolta, durante una ricerca, si rendono disponibili nuove informazioni sul farmaco in studio che possono modificare il rapporto rischio/beneficio di una terapia. Se ciò dovesse accadere, Le forniremo il prima possibile le nuove informazioni che potrebbero influire sulla Sua volontà di proseguire lo studio.
Lei è comunque libero/a di interrompere la partecipazione alla ricerca quando lo ritenga opportuno, senza dover fornire alcuna spiegazione; La invitiamo a informare tempestivamente il responsabile della ricerca, qualora ciò si verifichi, ma questo non influenzerà in alcun modo la qualità delle cure mediche di cui potrebbe aver bisogno in futuro.

Interazioni con farmaci, alimenti e sostanze chimiche (compresi alcool e stupefacenti)
Poiché alcuni medicinali/alimenti/sostanze chimiche (alcool e stupefacenti) potrebbero interferire con i farmaci in studio, con potenziali rischi per la salute, Le chiediamo di rivedere con il Medico Responsabile dello studio, tutti i farmaci che Lei assume regolarmente o occasionalmente, compresi i prodotti da banco, i preparati di erboristeria e gli integratori dietetici
Il Medico Responsabile verificherà la compatibilità di quanto Lei sta assumendo con i farmaci in studio e potrà suggerirLe dei cambiamenti. Qualora Lei non accettasse di interrompere l'assunzione di farmaci o sostanze incompatibili con i farmaci in studio, sarebbe esclusa/o dalla ricerca.
Non sono state segnalate interazioni significative tra gli inibirori dell'aromatasi (sia steroidei che non-steroidei) ed i farmaci più comunemente utilizzati, ma deve comunque essere usata cautela nell'assunzione concomitante di farmaci come rifampicina, fenitoina, carbamazepina e di preparati a base di erbe contenenti hypericum perforatum (Erba di San Giovanni) che potrebbe teoricamente ridurre l'efficacia degli inibitori dell'aromatasi. Infine, gli inibitori dell'aromatasi non devono essere assunti insieme a tamoxifen o a medicinali contenenti estrogeni, poiché questi annullerebbero la loro azione farmacologica.

Le chiediamo inoltre di avvisare preventivamente il Medico responsabile dello studio riguardo alla Sua intenzione di assumere un qualsiasi nuovo farmaco/sostanza.

Lei è obbligato/a a partecipare a questa ricerca?
La Sua decisione di partecipare a questa ricerca è completamente **libera e volontaria**.
Lei è libero/a di decidere di ritirarsi dalla ricerca in qualsiasi momento e senza dare alcuna spiegazione, senza alcuna penalità o perdita di benefici.
I dati raccolti fino al momento del ritiro potranno essere comunque utilizzati e non saranno raccolti ulteriori dati.

Contraccezione, Gravidanza e Allattamento
Uomini e donne sessualmente attivi devono usare efficaci metodi di contraccezione durante lo studio e per i 6 mesi successivi all'ultima assunzione dell'ultimo trattamento sperimentale.
Poiché chemioterapici e farmaci ormonali possono arrecare danni al feto o, in caso di allattamento, al bambino, se Lei è una paziente in età fertile **deve impegnarsi a non iniziare una gravidanza** per tutta la durata dello studio e per i 6 mesi successivi all'ultima assunzione dell'ultimo trattamento sperimentale, e a **non allattare per il medesimo periodo**.
Poiché i farmaci LHRH analoghi, pur bloccando il ciclo mestruale, non sono sufficientemente sicuri come anticoncezionali, è necessario utilizzare metodi contraccettivi più efficaci (per es. astinenza completa, se ciò è in linea con le Sue preferenze; metodi combinati di barriera e sistemi intrauterini). La pillola anticoncezionale (estro-progestinica) risulta invece controindicata in pazienti con tumore mammario.
Si consiglia quindi di intraprendere metodi contraccettivi altamente efficaci come:
- contraccezione di barriera doppia
- spirale contraccettiva (IUD)
- sterilizzazione tubarica
- vasectomia del partner
- astinenza

Per qualsiasi domanda in merito ai metodi contraccettivi, La preghiamo di rivolgersi al Medico Responsabile dello studio.
Se Lei è una donna in età fertile, all'inizio dello studio verrà sottoposta a un test di gravidanza. Nel caso si verificasse una gravidanza o il sospetto di gravidanza, dovrà informarne immediatamente il Medico Responsabile dello studio.

Quali rischi correrà se parteciperà a questa ricerca?
I farmaci utilizzati nello studio sono in commercio da anni in Italia ed ampiamente utilizzati. Se ne conoscono pertanto dettagliatamente gli effetti collaterali, anche quelli molto rari.
Lei deve informare della Sua partecipazione allo studio il personale sanitario che La prende in cura, specialmente se sta per sottoporsi ad un trattamento chirurgico, dentario, o a un qualsiasi altro trattamento.

Le viene consegnata una lettera destinata al Suo medico curante. La lettera contiene tutte le informazioni relative alla Sua partecipazione allo studio, in particolare l'indicazione degli effetti delle terapie che riceverà durante la sperimentazione, dei farmaci che non deve assumere e dei trattamenti medici ai quali non deve sottoporsi, in quanto incompatibili o sconsigliati.

La invitiamo, nel Suo interesse e a tutela della Sua salute, a: 1) consegnare la lettera al Suo medico curante; 2) informare i medici che La seguono nella sperimentazione di ogni farmaco o prodotto omeopatico o fitoterapico che intendesse assumere e di ogni trattamento medico al quale intendesse sottoporsi.

Qualora Lei omettesse di fornire tali indispensabili informazioni, né il Suo medico curante, né i medici che La seguono nella sperimentazione potranno essere considerati responsabili per i danni derivanti dalla incompatibilità tra la sperimentazione e ogni diverso trattamento medico. La avvertiamo inoltre che tali danni potrebbero non essere coperti dall'assicurazione stipulata al momento della Sua partecipazione allo studio.

Durante lo studio non deve effettuare donazioni di sangue e/o di emocomponenti.

Se, in qualsiasi momento, Lei dovesse sperimentare un effetto collaterale, bisognerà informarne al più presto il Medico Responsabile della ricerca senza aspettare la prossima visita programmata, al fine di poter prendere, quando necessario, dei provvedimenti.

Durante la partecipazione a questa ricerca Lei potrebbe essere sottoposto/a ai seguenti esami/indagini strumentali che presentano questi rischi:
- TAC: l'esame comporta l'esposizione a radiazioni ionizzanti, che sono potenzialmente dannose per la salute, perché potrebbero nel tempo causare i tumori; tuttavia questa evenienza è molto rara, e passa del tutto in secondo piano in pazienti già affetti da tumore, nei quali è molto più iportante valutare l'efficacia e quindi la correttezza della terapia del tumore presente, eseguendo periodiche valutazioni dell'andamento della malattia con esami diagnostici come la TAC, piuttosto che preoccuparsi di un ipotetico secondo tumore che potrebbe verificarsi anni dopo.
- Scintigrafia ossea e PET: tali esami prevedono la somministrazione endovenosa di sostanze radioattive, che emettono radiazioni per circa 24 ore; la dose di radiazioni ricevute da chi sisottopone all'esame è equivalente circa a quella che si assume quando si esegue una TAC; una piccola quota di radiazioni può però raggiungere anche persone vicine alla/al paziente, per cui è raccomandato che ella/egli eviti il contatto con donne in gravidanza e bambini piccoli, fintanto che la radioattività non sia scomparsa dall'organismo (circa 24 ore).
- Risonanza magnetica: questa non espone a radiazioni ionizzanti; non possono sottoporsi a risonanza magnetica i portatori di pacemaker cardiaco o di neurostimolatori perché il campo magnetico o le onde prodotte dall'apparecchiatura potrebbero alterarne il funzionamento; più in generale non possono sottoporsi a risonanza magnetica persone portatrici di apparecchiature metalliche (esempio pacemaker, protesi, chiodi e viti applicate in ortopedia, clips vascolari) o schegge metalliche (in seguito a incidenti o ad

attività lavorative) nel corpo, poiché queste potrebbero muoversi in seguito all'esposizione al forte campo magnetico e provocare danni ai tessuti di vario genere. La maggior parte delle apparecchiature mediche impiantate attualmente è compatibile con la risonanza magnetica, ma quelle più vecchie possono non esserlo: è sempre necessario quindi verificare la compatibilità discutendone con il medico e/o consultando il materiale informativo delle apparecchiature.
- Ecografia: non comporta rischi per la salute.

Il farmaco in studio Le apporterà dei benefici?
I risultati di ricerche già condotte ci fanno pensare che il trattamento sperimentale possa migliorare la Sua salute, però non ci sono garanzie assolute in tal senso, quindi non possiamo garantirLe né prometterLe effetti benefici certi.
Tuttavia Lei potrà giovarsi di un attento monitoraggio delle Sue condizioni fisiche, e avrà la possibilità di dare il Suo contributo ad una ricerca scientifica che in futuro potrebbe aiutare persone che hanno la Sua stessa malattia.

Per la sua partecipazione allo studio è previsto un compenso?
Non è previsto alcun compenso per la Sua partecipazione allo studio. Allo stesso tempo Lei non dovrà pagare nulla per le visite, gli esami ed i farmaci previsti da questo studio.

Chi ha esaminato lo studio?
Questo studio è stato approvato dal Comitato Etico IRST IRCCS AVR, ossia il comitato che garantisce la tutela dei diritti dei soggetti umani presso IRST IRCCS ed Area Vasta Romagna, promotore e centro coordinatore dello studio.
Lo studio è stato inoltre approvato dal Comitato Etico _____
a cui fa riferimento il Medico Responsabile dello studio presso il Centro Partecipante di
_____.

La ricerca ha una copertura assicurativa?
Il Promotore che ha commissionato lo studio ha stipulato apposita polizza di Responsabilità Civile verso Terzi con la società Lloyd's che prevede il risarcimento dei danni causati durante la sperimentazione in conformità al protocollo.
La polizza assicurativa garantisce la copertura dei danni da responsabilità civile derivante dalla sperimentazione stessa fino a € 1.000.000 per paziente, non copre il valore eccedente il massimale, ed è operante esclusivamente per i danni manifestati entro 24 mesi dalla conclusione della sperimentazione e per richieste di risarcimento presentate entro e non oltre 36 mesi dalla conclusione della sperimentazione stessa. Tale limitazione non inficia comunque il diritto del soggetto danneggiato ad ottenere il risarcimento da parte del responsabile dell'eventuale danno. Sono esclusi dalla copertura della polizza assicurativa:
- i danni causati da sperimentazioni non regolarmente autorizzate e/o svolte in maniera difforme da quanto autorizzato

- i danni che non siano in relazione causale nei termini stabiliti dalla legge 211/2003 con la sperimentazione assicurata
- i reclami dovuti al fatto che la formulazione farmaceutica soggetta a sperimentazione non realizza gli scopi curativi previsti
- i danni congeniti o malformazioni, provocate in donne incinte partecipanti alla sperimentazione
- i danni genetici infermità genetiche e/o ereditarie
- i danni nucleari di qualsiasi tipo
- i reclami dovuti ad immunodeficienza acquisita da HIV o ad errata diagnosi di tale sindrome
- i danni derivanti dai seguenti prodotti farmaceutici: anticoncezionali ormonali, oggetto del protocollo sperimentale; stilbestrol/D.E.S.; predimodone; fluoxetine; phenylropanolamine; methylphenidate; troglitazone; gemfibrozil; cerivastatin; isotretinoin.

Si specifica che per tutte le procedure secondo pratica clinica si fa riferimento alla copertura assicurativa aziendale del Centro Partecipante presso cui è in cura.

Si precisa che per tutta la durata dello studio saranno operative idonee coperture assicurative.

Per qualsiasi domanda riguardante la garanzia di risarcimento e le cure mediche e per qualunque disturbo o danno Lei dovesse ritenere correlato alla sperimentazione La preghiamo di contattare:

Nome e Cognome: _____ Tel nr.: _____

Aspetti etici

La ricerca cui Le è stato proposto di partecipare, incluso il presente modulo informativo, è stata esaminata ed approvata dal Comitato Etico di questa struttura. Lo studio è stato progettato e verrà condotto in conformità agli standard etici internazionali e nazionali sulla ricerca biomedica con esseri umani: in particolare, alle revisioni correnti della dichiarazione di Helsinki dell'Associazione Medica Mondiale sui "Principi etici per la ricerca medica che coinvolge soggetti umani" e delle Norme di Buona Pratica Clinica (ICH/GCP) della Unione Europea sulla sperimentazione dei medicinali laddove applicabili; alla Convenzione del Consiglio d'Europa per la protezione dei diritti dell'uomo e della dignità dell'essere umano nell'applicazione della biologia e della medicina (Convenzione di Oviedo del 04/04/1997); ai contenuti dei codici italiani di deontologia delle professioni sanitarie e della specifica normativa nazionale vigente in tema di studi clinici. Lei può segnalare qualsiasi fatto ritenga opportuno evidenziare, relativamente allo studio che La riguarda, al Comitato Etico di questa struttura. In nessun caso l'approvazione del Comitato Etico deve essere da Lei considerata come un incoraggiamento a partecipare a questo studio.

Come sarà protetta la sua riservatezza?

Il Suo consenso alla partecipazione a questa ricerca significa che Lei autorizza l'uso dei dati personali e sensibili contenuti nella Sua cartella clinica, in maniera anonima per esclusivi motivi di ricerca. Questi dati potranno anche essere confrontati a scopo statistico con dati analoghi provenienti da altre fonti epidemiologiche o cliniche. Tutte le informazioni (personali, cliniche) raccolte durante questa ricerca sono confidenziali e verranno trattate nel rispetto della normativa vigente (D.Lgs. 30.06.03 n.196 – Codice in materia di protezione dei dati personali).

Alla fine della ricerca i risultati potranno essere pubblicati ma la sua identità resterà anonima.

La informiamo inoltre che la documentazione clinica originale che la riguarda potrà essere visionata dal Promotore dello studio o da suoi rappresentanti, dal Comitato Etico o dalle Autorità regolatorie di governo, quali ad esempio il Ministero della Salute italiano, la Food and Drug Administration (Stati Uniti), per verificare che le informazioni riportate sui documenti dello studio siano corrette e vere.

Lei può decidere di NON dare il Suo consenso all'utilizzo dei suoi dati, ma in tal caso NON potrà partecipare alla ricerca. Può anche decidere di ritirare il Suo consenso a partecipare in qualsiasi momento, tuttavia le informazioni raccolte sino al Suo ritiro potranno comunque essere utilizzate come dati dello studio.

Lei ha il diritto di vedere le informazioni personali che La riguardano, ad es. nome, indirizzo, e ha il diritto di correggere tali dati se necessario.

In che modo saranno conservati i dati?

Se Lei è d'accordo, i dati riguardanti la Sua malattia raccolti per lo studio IRST174.19 saranno conservati in un archivio elettronico presso:

Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS
Via Piero Maroncelli 40, 47014 Meldola (Fc).

Tale archivio è realizzato secondo le disposizioni del garante per la tutela dei dati personali (decreto legislativo n.196/2003 – Codice in materia di protezione dei dati personali) e, quando applicabile, l'Autorizzazione al trattamento dei dati sensibili.

Con il Suo specifico consenso scritto le informazioni raccolte saranno trattate attraverso l'ausilio di strumenti informatici nel rispetto dei principi di necessità ed indispensabilità.

Gli strumenti informatici sono progettati in modo tale da ridurre al minimo l'utilizzo di dati personali e dati identificativi, per escluderne il trattamento quando le finalità perseguite nei singoli casi possono essere realizzate mediante dati anonimi o modalità opportune che permettano di identificare l'interessato solo in caso di necessità.

I Suoi dati saranno utilizzati per l'avanzamento delle conoscenze nel campo delle cure oncologiche. Questa attività sarà sviluppata nel rispetto delle disposizioni sulla tutela dei dati personali ed in accordo con le regole etiche vigenti. I Suoi dati genetici saranno trattati ai sensi dell'Autorizzazione del Garante della Privacy in vigore.

Le assicuriamo inoltre che le informazioni fornite ed i risultati della ricerca saranno trattati confidenzialmente e secondo gli obblighi deontologici relativi alle singole figure professionali previste dall'autorizzazione del Garante per la protezione dei dati personali.

I dati raccolti potranno essere comunicati o trasferiti ai soggetti partecipanti allo studio, sempre limitatamente alle informazioni prive di dati identificativi, solo per scopi scientifici direttamente collegati a quelli per i quali sono stati originariamente raccolti e chiaramente determinati per iscritto nella richiesta dei dati. Il soggetto richiedente si impegnerà a non trattare i dati per fini diversi da quelli indicati nella richiesta e a non comunicarli o trasferirli ulteriormente a terzi.

Tutte le ricerche sono state avviate previa l'autorizzazione del Comitato etico IRST IRCCS AVR a cui fa riferimento il Promotore dello studio, per assicurare il rispetto delle disposizioni e regole menzionate.

I dati saranno conservati per un periodo di 15 anni dopo la chiusura dello studio oppure fino al Suo eventuale ritiro del consenso, sotto la responsabilità del Centro Coordinatore, a meno di disposizioni diverse da parte Sua.

I dati saranno raccolti in forma anonima e non saranno in alcun modo resi pubblici o trasferiti ad altri enti se non in forma aggregata.

Sarà assicurata la confidenzialità dei dati che saranno trattati secondo le normative vigenti e nel rispetto dei Suoi diritti.

Cosa significa dare il consenso informato?

Se acconsente a partecipare alla ricerca, dovrà firmare l'allegato modulo di consenso. La firma di questo modulo non Le toglierà in alcun modo i Suoi diritti; essa viene richiesta soltanto al fine di garantire che Lei sia stata/o informata/o in modo completo sullo studio, che ne abbia capito lo scopo ed il Suo coinvolgimento.

Prima di firmare verifichi, per cortesia, se Le è tutto chiaro riguardo allo studio e a quello che dovrà fare; se Le restano dei dubbi non abbia timore di richiedere ulteriori spiegazioni.

Se ha qualsiasi domanda, incluse quelle inerenti questo studio o riguardanti i Suoi diritti, o se crede di essere stata/o danneggiato/a in qualunque modo partecipando a questo studio, La preghiamo di farlo presente al Medico responsabile della ricerca ora oppure nel corso dello studio, contattandolo al numero telefonico qui di seguito riportato. Il Medico responsabile della ricerca ed il personale che lo assiste nella conduzione della ricerca sarà lieto di rispondere a qualsiasi Sua domanda. Nessuna pressione verrà fatta su di Lei affinché partecipi a questo studio.

Se è d'accordo, il Medico responsabile della ricerca informerà il Suo Medico curante della Sua partecipazione a questa ricerca.

Lei ha il diritto in ogni momento di fare domande sulla ricerca e di richiedere comunicazioni ed informazioni circa lo studio al: Dr./Prof. _____ presso l'Ospedale/Reparto/N. tel. _____.

Libero arbitrio – Diritti dell'interessato – Revoca del consenso

La Sua collaborazione è libera e volontaria. Pertanto Lei è libera/o di revocare, in ogni momento il Suo consenso. Le richieste di esercizio dei diritti previsti dal Codice a favore dell'interessato (art. 7 e ss D.Lgs. 30 giugno 2003, n. 196; ad esempio, avere comunicazione dei dati trattati che La riguardano, conoscerne l'origine, la logica, le finalità e le modalità su cui si basa il trattamento; ottenerne l'aggiornamento, la rettifica o l'integrazione) possono essere rivolte al medico di riferimento che le ha proposto di partecipare a questo studio.

Si sottolinea che il protocollo sperimentale è stato redatto in conformità alle Norme di Buona Pratica Clinica e nel rispetto dei principi etici stabiliti nelle dichiarazioni internazionali.
La ringraziamo anticipatamente del Suo aiuto per questa ricerca.

MODULO DI CONSENSO INFORMATO

Sigla di identificazione dello studio: IRST 174.19
Titolo dello studio: Studio clinico randomizzato di confronto tra chemio-endocrinoterapia concomitanti e chemioterapia seguita da endocrinoterapia come trattamento di prima linea del carcinoma mammario metastatico luminale B.
Data e Versione N°: 03/03/2017 – Versione Finale

Sperimentatore Principale: _____
Promotore dello studio: Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS

L'INFORMAZIONE AL PAZIENTE IN VIRTÙ DELLA PROPEDEUTICITÀ DI TALE FASE DOVRÀ ESSERE FORNITA IN UN MOMENTO PRECEDENTE E FORMALMENTE DISTINTO DAL RECEPIMENTO DEL CONSENSO.

Io sottoscritta/o _____, firmando il presente documento:

1. Confermo di aver ricevuto dal Dr._____ informazioni scritte (foglio informativo per il paziente studio IRST174.19, del 03/03/2017 versione finale) e verbali sullo studio in oggetto e sulle previsioni di rischi e benefici connessi alla sperimentazione, e di aver ricevuto copia del foglio informativo e del presente modulo di consenso firmato e datato.
2. Confermo di avere avuto il tempo necessario per porre tutte le domande per me importanti relative allo studio. Sono soddisfatta/o di tutte le risposte che mi sono state date.
3. Sono consapevole che la mia partecipazione a questo studio è volontaria e che sono libera/o di ritirami dalla ricerca in ogni momento, senza darne ragione e senza incorrere in problemi o svantaggi per la mia assistenza medica futura.
4. Sono consapevole che il Promotore della ricerca ha stipulato apposita polizza di Responsabilità Civile che prevede il risarcimento di danni o lesioni causate dall'esecuzione della ricerca.
5. Sono consapevole della necessità, diretta a garantire la migliore tutela della mia salute, di informare il mio medico curante della sperimentazione alla quale accetto di partecipare, consegnandogli la lettera a lui indirizzata; nel caso decida di non informarlo o ometta di farlo, né il mio medico curante né i medici che mi seguono nella sperimentazione saranno considerati responsabili per i danni che possano derivare dall'incompatibilità tra la sperimentazione e qualunque altro diverso trattamento medico o farmaco o prodotto omeopatico o fitoterapico io abbia assunto. Inoltre sono consapevole che tali danni

potrebbero non essere coperti dall'assicurazione stipulata al momento della mia decisione di partecipare allo studio.

- ▪ **autorizzo**
- ▪ **non autorizzo**

a fornire al mio Medico di Medicina Generale notizie sulla mia partecipazione a questo studio clinico.

6. ▪ **desidero**
   ▪ **non desidero**

ricevere informazioni da parte del Medico Responsabile dello studio sui risultati della ricerca.

7. ▪ **desidero**
   ▪ **non desidero**

ricevere informazioni da parte del Medico Responsabile dello studio sugli eventuali risultati accidentali che dovessero derivare dalla ricerca.

8. Sono consapevole del fatto che la mia partecipazione allo studio comporta la raccolta e l'elaborazione dei miei dati personali, compresi quelli sensibili. Tutti i dati verranno comunque raccolti in forma anonima, ai sensi del Decreto Legislativo n. 196 del 30/06/2003 in materia di protezione dei dati personali, e solo per quanto risulterà necessario ai fini dello studio sopra citato.

9. Sono stata/o informata/o dell'approvazione dello studio da parte del Comitato Etico Indipendente locale, a tutela della correttezza della sperimentazione e dei diritti del malato.

Il Dr _____ mi ha consegnato copia firmata e datata di questo modulo di consenso informato e del foglio informativo.

Acconsento a partecipare a questo studio.

Nome e Cognome del Soggetto (in stampatello):

_____

Data_____/_____/_____ Firma del Soggetto _____
(da apporre da parte del Soggetto)

Nome e Cognome del Testimone Imparziale* (in stampatello): _____

Data_____/_____/_____ Firma del Testimone Imparziale _____

Nome e Cognome del Testimone Imparziale* (in stampatello): **_____**

Data_____/_____/_____ Firma del Testimone Imparziale _____

* una persona che sia indipendente dallo studio, che non possa essere influenzata in alcun modo dalle persone coinvolte nello studio, che partecipi alla procedura di informazione del paziente se il paziente non è in condizione di leggere e che legga il consenso informato e ogni altra informazione scritta fornita al paziente partecipante

DA COMPLETARE DA PARTE DEL MEDICO DELLO STUDIO CHE HA OTTENUTO IL CONSENSO
Confermo di aver fornito al paziente esaurienti spiegazioni circa la natura, lo scopo, la durata e i possibili rischi connessi con lo studio in argomento e di avergli consegnato una copia del foglio informativo ed una copia datata e firmata del modulo di consenso.

Nome e Cognome del Medico dello studio che ha ottenuto il consenso
(in stampatello)_____

Data_____/_____/_____

Firma del Medico che ha ottenuto il consenso _____

FOGLIO INFORMATIVO PER IL PAZIENTE

Sigla di identificazione dello studio: IRST174.19

Titolo dello studio: Studio clinico con randomizzazione sequenziale adattativa a gruppi di confronto tra chemioterapia + endocrinoterapia verso inibitori delle chinasi ciclina-dipendenti 4 e 6 (CDK4/6) + endocrinoterapia nel carcinoma mammario avanzato a recettori ormonali positivi e HER2-negativo..

Data e Versione N°: 09/11/2018 – Emendamento 1.0

Sperimentatore Principale: _____

Promotore dello studio: Istituto Scientifico Romagnolo per lo Studio e la Cura dei
Tumori (IRST) IRCCS
Via Piero Maroncelli, 40/42
47014 Meldola (FC)

Nome e Cognome del paziente: _____

Gentile Sig.ra/Sig.re, le informazioni contenute nella scheda seguente intendono essere esaurienti e sono quindi DETTAGLIATE e COMPLESSE. Le chiediamo di accettare la partecipazione allo studio SOLO dopo avere letto con attenzione la scheda ed avere avuto un colloquio ESAURIENTE con il medico sperimentatore che le dovrà dedicare il TEMPO NECESSARIO per una comprensione di ciò che le viene proposto.

Introduzione
Gent. Sig.ra/re, Le viene chiesto di partecipare ad una ricerca clinica dal titolo "*Studio clinico con randomizzazione sequenziale adattativa a gruppi di confronto tra chemioterapia + endocrinoterapia verso inibitori delle chinasi ciclina-dipendenti 4 e 6 (CDK4/6) + endocrinoterapia nel carcinoma mammario avanzato a recettori ormonali positivi e HER2-negativo.*"

Prima di decidere se partecipare o meno, è importante che Lei venga informato/a dei motivi per i quali la ricerca viene condotta e cosa comporterà per Lei la partecipazione alla ricerca. Si prenda tutto il tempo necessario per leggere queste informazioni attentamente e, se lo desidera, ne discuta con amici, parenti e col Suo medico di famiglia.
Non esiti a fare domande se qualcosa (ad esempio qualche termine medico o espressione) non Le è chiaro o se desidera avere maggiori informazioni.

Chi sta conducendo questa ricerca e quanti pazienti saranno coinvolti?
Questa ricerca coinvolgerà al massimo 150 pazienti in Italia e viene condotta presso l'Istituto

Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS (promotore e centro coordinatore), sotto la responsabilità del dott. Andrea Rocca e dal Centro di _____, sotto la responsabilità di _____.

Perché Le è stato chiesto di partecipare a questo studio?

Il Medico che la cura Le ha proposto di partecipare a questa ricerca in quanto Lei risulta affetta/o da carcinoma (un tipo di tumore) della mammella localmente avanzato (ossia diffuso dalla mammella ai tessuti adiacenti quali la cute, i linfonodi, o le strutture della parete toracica) non suscettibile di trattamento chirurgico o radioterapico radicali (ossia in grado di eradicare completamente la malattia), oppure metastatico (ossia diffuso ad organi distanti dalla mammella, quali scheletro, fegato, polmoni, o altri), quindi non trattabile con chirurgia o radioterapia, ma bensì principalmente mediante l'uso di farmaci. In particolare si tratta di un carcinoma cosiddetto "luminale", che è caratterizzato dalla presenza di "recettori per gli estrogeni" (o recettori estrogenici), molecole a cui si legano gli estrogeni che, legandosi ai recettori estrogenici, stimolano la crescita dei carcinomi mammari luminali. Pertanto una modalità efficace di trattamento dei carcinomi mammari luminali consiste nell'impiego di farmaci che bloccano la produzione di estrogeni nell'organismo oppure che impediscono agli estrogeni di legarsi al recettore estrogenico. Genericamente, le terapie che agiscono sugli ormoni vengono indicate con il termine di "terapie ormonali", o con quello equivalente di "terapie endocrine" o "endocrinoterapia".

Nelle donne in pre-menopausa gli estrogeni vengono prodotti principalmente dalle ovaie e la loro produzione può essere bloccata mediante farmaci chiamati "agonisti dell'ormone di rilascio delle gonadotropine" (GnRH agonisti), detti anche "agonisti dell'ormone di rilascio dell'ormone luteinizzante" (LHRH agonisti). Questi farmaci bloccano il ciclo mestruale e la produzione di estrogeni da parte delle ovaie.

Nelle donne in post-menopausa (e in quelle in pre-menopausa in trattamento con LHRH agonisti) la produzione di estrogeni avviene da parte di altri tessuti, in primo luogo il tessuto adiposo, e può essere bloccata mediante farmaci chiamati "inibitori dell'aromatasi" (aromatasi è il nome dell'enzima che favorisce la produzione degli estrogeni).

Pertanto, una adeguata soppressione della produzione di estrogeni si ottiene mediante un inibitore dell'aromatasi nelle donne in post-menopausa e mediante la combinazione di LHRH agonista + inibitore dell'aromatasi nelle donne in pre-menopausa. L'inibitore dell'aromatasi è inoltre efficace come terapia del carcinoma mammario in soggetti maschi, nei quali viene utilizzato da solo o in combinazione con LHRH agonista.

Un altro farmaco efficace è il fulvestrant, che si lega al recettore estrogenico provocandone la distruzione e impedendo il legame degli estrogeni al recettore stesso. Fulvestrant viene utilizzato da solo nelle pazienti in post-menopausa e in associazione ad LHRH agonisti nelle pazienti in pre-menopausa.

Recentemente si sono resi disponibili farmaci, detti inibitori delle chinasi ciclina-dipendenti 4 e 6 (CDK4/6 inibitori), in grado di potenziare l'efficacia delle terapie ormonali, quando associati a inibitore dell'aromatasi o a fulvestrant.

Mentre le terapie ormonali, da sole o in associazione a CDK4/6 inibitori, sono l'opzione di prima scelta nei tumori mammari luminali più indolenti, non è al momento noto quale sia il trattamento più indicato nei tumori luminali che presentino una o più caratteristiche di aggressività, quali un basso livello di recettori estrogenici, un breve intervallo di tempo tra l'intervento chirurgico sul tumore primitivo e la ricomparsa della malattia, un alto indice proliferativo, una bassa o assente espressione dei recettori per progesterone, o il coinvolgimento importante di organi (quali fegato o polmoni) e/o la ridotta funzionalità di uno o più organi. In questi casi i trattamenti che si possono utilizzare sono la combinazione di endocrinoterapia + CDK4/6 inibitori, o una chemioterapia.

Esistono inoltre studi di laboratorio (e anche alcuni studi clinici su pazienti) che mostrano come l'utilizzo concomitante, simultaneo, di chemioterapia e terapia endocrina possa risultare più efficace rispetto alla somministrazione della chemioterapia da sola.

Questo studio si propone pertanto di confrontare il trattamento con endocrinoterapia + CDK4/6 inibitori verso il trattamento con chemioterapia + endocrinoterapia in pazienti con tumori luminali che presentino una o più caratteristiche di aggressività. Per effettuare questo confronto, le/i pazienti vengono suddivisi in due gruppi, un gruppo che riceve endocrinoterapia + CDK4/6 inibitore e uno che riceve chemioterapia + endocrinoterapia. In quest'ultimo gruppo, il/la paziente può ricevere una chemioterapia per 4-6 mesi (eccetto che in caso di chemioterapie molto ben tollerate, per cui il Suo oncologo potrebbe decidere di continuare il trattamento più a lungo) e poi proseguire con una terapia ormonale di mantenimento, oppure, a discrezione dell'oncologo referente, può ricevere la terapia ormonale in concomitanza alla chemioterapia per 4-6 mesi (o più a lungo nel caso di chemioterapie molto ben tollerate) e proseguire successivamente con la sola terapia ormonale di mantenimento.

Per poter fare un confronto obiettivo tra i due trattamenti (endocrinoterapia + CDK4/6 inibitori e chemioterapia + endocrinoterapia) è necessario che i pazienti messi in ciascuno dei due gruppi non siano selezionati in base a caratteristiche che possano influenzare l'esito del trattamento (ad esempio, mettere tutti i pazienti con malattia più aggressiva in un gruppo e tutti quelli con malattia più indolente nell'altro favorirebbe nei risultati il trattamento attribuito al secondo gruppo, in maniera artificiosa, provocando quella che in termini tecnici viene definita una "distorsione" nei risultati dello studio). Pertanto si rende necessario utilizzare, nell'attribuzione dei pazienti ai due gruppi di trattamento, un processo di randomizzazione adattativa, che consiste nell'attribuire i pazienti ai gruppi in maniera casuale: per ogni paziente incluso nello studio viene "estratto a sorte" il gruppo in cui quel paziente verrà inserito. Il processo di randomizzazione adattativa è svolto tramite un programma di computer, ma è sostanzialmente equivalente ad effettuare un'estrazione da un'urna contenente palline rosse e blu in proporzione diversa ed attribuire il paziente ad un gruppo se è estratta una pallina rossa e all'altro gruppo se la pallina estratta è blu. Le probabilità di assegnazione ad ognuno dei 2 gruppi (equivalenti nell'esempio alle proporzioni di palline dei 2 colori nell'urna) saranno calcolate in tre distinti istanti temporali durante il corso della sperimentazione. Le probabilità di assegnazione ai due gruppi di trattamento che saranno calcolate in questi istanti temporali andranno a favorire leggermente il trattamento che risulta più promettente sulla base dei dati

fino ad allora raccolti. Come ulteriore tutela sono oltretutto previste due analisi intermedie che permetteranno di valutare se i due gruppi di trattamento diano risultati analoghi, in caso contrario lo studio verrà interrotto e verrà dichiarato che uno dei due trattamenti porta a risultati migliori.

Il fatto che il gruppo di trattamento venga scelto "a caso" non dovrebbe essere motivo di preoccupazione, dal momento che non è ad oggi noto quale sia il trattamento migliore, ma sia l'endocrinoterapia + CDK4/6 inibitori che la chemioterapia + endocrinoterapia sono terapie di comprovata efficacia e vengono normalmente utilizzate in sequenza nello stesso paziente. In particolare, se il trattamento che Le è stato assegnato non si dimostrasse sufficientemente efficace, o perdesse efficacia dopo un periodo di buona attività, Lei potrà ricevere il trattamento corrispondente all'altro braccio dello studio (a meno che il Suo oncologo non ritenga maggiormente indicato un trattamento ancora diverso). L'ipotesi alla base di questo studio clinico è che nei tumori più aggressivi sia più efficace la chemioterapia + endocrinoterapia, mentre in quelli meno aggressivi sia adeguata la endocrinoterapia + CDK4/6 inibitori, ma lo studio mira in particolare ad individuare le caratteristiche cliniche e biologiche tumorali che predicano una migliore risposta a uno o all'altro dei trattamenti.

Qual è il farmaco che viene sperimentato in questa ricerca?
La scelta del tipo di chemioterapia è a discrezione del Suo oncologo, il quale sceglierà il o i chemioterapici che ritiene più indicati in base alle caratteristiche della Sua malattia e alle Sue condizioni generali. Si tratta comunque e sempre di farmaci già di uso corrente, dei quali sono ben noti efficacia e tossicità.

La scelta del trattamento ormonale è ristretta all'uso degli inibitori dell'aromatasi e al fulvestrant (ai quali andrà associato un LHRH agonista nelle donne in pre-menopausa ed eventualmente negli uomini). Esistono 3 farmaci appartenenti alla classe degli inibitori dell'aromatasi: anastrozolo, letrozolo, ed exemestane; i primi due sono di tipo "non steroideo" e l'ultimo di tipo "steroideo" (questi termini si riferiscono alla struttura chimica di tali farmaci). L'unico vincolo nella scelta di quale inibitore dell'aromatasi assumere nello studio è che andrà utilizzato un inibitore di tipo steroideo se vi è già stata una progressione tumorale in corso di precedente terapia con un inibitore non steroideo e viceversa. Anche questi sono farmaci già di uso corrente e di cui sono noti efficacia e tossicità.

I CDK4/6 inibitori che hanno completato tutti gli stadi di sviluppo clinico sono 3: palbociclib, ribociclib e abemaciclib. Essi hanno caratteristiche farmacologiche simili e la scelta verrà fatta dal Suo oncologo sulla base della sua situazione di malattia e dei precedenti trattamenti.

I farmaci utilizzati in questo studio sono perciò già registrati per la sua malattia e non sono pertanto trattamenti "sperimentali". Solo la somministrazione concomitante di chemioterapia ed endocrinoterapia (che non è obbligatoria ed è a discrezione del Suo oncologo) non è considerata uno standard, sebbene sia spesso utilizzata in clinica.

Qual è lo scopo della ricerca?
Lo scopo di questa ricerca è confrontare l'efficacia e la tossicità del trattamento con endocrinoterapia + CDK4/6 inibitori rispetto al trattamento con chemioterapia +

endocrinoterapia. L'efficacia in questo studio viene valutata in termini di capacità del trattamento di tenere sotto controllo la malattia tumorale, ossia di evitare un peggioramento della neoplasia (in termini tecnici il peggioramento è indicato come "progressione" di malattia). Ripetendo una TAC circa ogni 3 mesi dall'inizio del trattamento, si valuta ogni volta se la malattia sia migliorata (si parla allora di "risposta" di malattia, che può essere parziale o completa), o stabile/invariata (si parla di "stabilità" di malattia), o peggiorata ("progressione"). Sia la risposta che la stabilità di malattia stanno a indicare che il trattamento sta efficacemente contrastando la malattia, mentre la progressione di malattia indica che il trattamento non è efficace o ha esaurito la sua efficacia. Pertanto, per ogni paziente si misurerà il tempo intercorso tra l'inizio del trattamento e il riscontro di una progressione di malattia, e questo rappresenterà il tempo durante il quale la malattia è rimasta controllata dal trattamento (in termine tecnico definito come "intervallo libero da progressione di malattia"). Calcolando una sorta di tempo medio libero da progressione di malattia per ognuno dei due gruppi di trattamento e confrontando tale tempo medio tra i due gruppi, si vedrà quale trattamento risulterà più efficace.

Per fare questo, dopo aver valutato l'estensione della malattia nell'organismo (mediante esami diagnostici come TAC e/o altri come risonanza magnetica, ecografia, scintigrafia ossea, PET) e aver controllato la funzionalità degli organi principali (midollo osseo, fegato, reni) con esami ematici, e dopo aver verificato che siano rispettati tutti i criteri di inclusione nello studio, avverrà la randomizzazione, ossia l'attribuzione casuale a uno dei due gruppi di trattamento. Iniziato il trattamento, la valutazione di efficacia avverrà, come detto sopra, mediante la ripetizione degli esami diagnostici (TAC e/o altri) circa ogni 3 mesi e la prosecuzione o meno del trattamento dipenderà dall'esito di tali esami. In caso di riscontro di "progressione" di malattia il trattamento verrà sospeso, mentre in caso di stabilità o risposta il trattamento verrà proseguito. In ogni caso la somministrazione della chemioterapia avverrà (a meno di effetti tossici che la rendano intollerabile) per un minimo di 4 mesi e fino a 6 mesi (la durata è variabile a seconda del tipo di trattamento e può anche superare i 6 mesi in caso di chemioterapie molto ben tollerate), dopo di che, una volta raggiunto il massimo beneficio (in termini di riduzione delle masse tumorali) ottenibile con quel trattamento, si potrà proseguire con la sola endocrinoterapia (ciò sia nel caso in cui l'endocrinoterapia venga iniziata in concomitanza alla chemioterapia, sia nel caso in cui si somministri la chemioterapia da sola e si prosegua poi con endocrinoterapia di mantenimento).).

La durata complessiva del trattamento non è definibile a priori ed il trattamento proseguirà fino ad esaurimento dell'efficacia (progressione di malattia) o al verificarsi di effetti tossici non tollerabili.

Allorché si verifichi una progressione della Sua malattia, dopo l'interruzione del trattamento a cui Lei è stata/o assegnata/o, si potrà passare all'altro trattamento previsto dal protocollo sperimentale se il Suo oncologo lo ritenesse appropriato.

Esistono dei trattamenti alternativi?
Se Lei non volesse partecipare alla ricerca che Le viene proposta, verrebbe curato/a o con endocrinoterapia + CDK4/6 inibitore o con chemioterapia + endocrinoterapia.

Se Lei deciderà di non partecipare a questa ricerca, il Medico Responsabile della ricerca valuterà con Lei il rapporto rischio/beneficio di tutte le possibili strategie terapeutiche attualmente disponibili per la cura della Sua malattia. Si senta libera/o di porre tutte le domande che desidera sulle diverse opzioni di trattamento.

A cosa andrà incontro se accetterà di partecipare alla ricerca?
Qualora Lei accetti di partecipare a questa ricerca, dovrà firmare il modulo di Consenso Informato e il Medico Responsabile della ricerca Le chiederà di sottoporsi ai seguenti esami/procedure:
- raccolta della storia clinica, inclusi i farmaci assunti per altre patologie*
- visita medica*
- registrazione parametri vitali (pressione, frequenza cardiaca, temperatura, oltre a peso e statura)*
- esami di laboratorio (sangue ed eventualmente urine)*
- TAC torace e addome completo con mezzo di contrasto (in caso di allergia al mezzo di contrasto TAC, si possono effettuare TAC torace senza mezzo di contrasto e Risonanza Magnetica dell'addome, possibilmente con mezzo di contrasto)
- eventuali altri esami diagnostici quando indicato, in base ai sintomi presenti o ad alterazione di esami di sangue (es. scintigrafia ossea, TAC o Risonanza Magnetica di altri settori)
- eventuale valutazione cardiologica con elettrocardiogramma, visita e/o ecocardiogramma se clinicamente indicato (in base alle patologie associate e al tipo di chemioterapia scelta)*
- valutazione della qualità di vita con appositi questionari: EORTC QLQ-C30 e QLQ-BR23.

Di questi esami, quelli indicati con l'asterisco farebbero comunque parte delle indagini necessarie per diagnosticare e trattare la patologia da cui è affetto/a. Inoltre, la TAC con mezzo di contrasto è anch'essa frequentemente utilizzata nella pratica clinica (al di fuori di studi clinici) sebbene possa talvolta essere efficacemente sostituita da radiografia del torace ed ecografia dell'addome.

Se Lei è una donna in età potenzialmente fertile, verrà effettuato anche un test di gravidanza da un campione di sangue o di urine, al fine di essere certi che Lei non sia incinta per non correre il rischio di esporre il nascituro agli effetti potenzialmente tossici del trattamento sperimentale.

Gli esami elencati sopra verranno ripetuti periodicamente nel corso dello studio: alcuni esami ematici prima di ogni ciclo di chemioterapia, gli esami per valutare l'estensione della malattia tumorale (TAC e/o altri visti sopra) circa ogni 3 mesi, gli accertamenti cardiologici in base alla situazione clinica.

Lo studio prevede anche la raccolta e la valutazione di campioni biologici di sangue e tessuto tumorale destinati ad indagini di biologia molecolare. Tali indagini hanno lo scopo di identificare dei fattori biologici (proteine, o mutazioni di geni) che aiutino a predire quali pazienti beneficeranno maggiormente del trattamento con endocrinoterapia + CDK4/6 inibitori piuttosto che con chemioterapia + endocrinoterapia. I risultati di questa ricerca potranno essere utili in futuro per il trattamento di pazienti affetti dalla Sua stessa patologia.
Si tratta in particolare di:

- utilizzo di un campione di tessuto tumorale derivato dal Suo tumore primitivo o, qualora disponibile, dalla biopsia di una metastasi, e conservato presso l'Anatomia Patologica del centro in cui ha eseguito la biopsia del tumore o l'intervento chirurgico di asportazione del tumore mammario
- un prelievo di sangue (10 ml) eseguito prima di iniziare il trattamento e ripetuto circa ogni 3 mesi per la durata dello studio
- un ulteriore prelievo di sangue (10 ml) eseguito prima di iniziare il trattamento, ripetuto dopo circa 2 mesi di trattamento e al termine della chemioterapia.

Se Lei accetterà di aderire, accetterà di fornire un campione di tessuto tumorale derivato dal Suo tumore già archiviato presso l'Anatomia Patologica del Centro, e di sottoporsi ai prelievi di sangue periodici sopracitati. La parte biologica è infatti integrante allo studio clinico ed obbligatoria per ciascun paziente che partecipa alla sperimentazione.

Farmaci dello studio - Trattamenti
Se il Medico responsabile della ricerca La riterrà idonea/o a partecipare alla ricerca, Lei riceverà i seguenti trattamenti:

- un trattamento chemioterapico, composto da uno o più farmaci chemioterapici, la cui scelta è a discrezione del suo oncologo e si baserà sulle caratteristiche della Sua malattia e sulle Sue condizioni generali. Si tratta comunque e sempre di farmaci già di uso corrente, dei quali sono ben noti efficacia e tossicità. Alcuni chemioterapici vengono somministrati per via endovenosa (e possono richiedere l'impianto di un catetere venoso), altri per bocca. La somministrazione avviene più spesso a "cicli", soprattutto per i farmaci utilizzati per via endovenosa, con somministrazioni ad esempio una volta ogni 3 settimane; altre volte le somministrazioni si fanno una volta alla settimana o talora, di solito per farmaci orali, tutti i giorni. Il tipo e l'entità degli effetti collaterali variano in base agli specifici farmaci utilizzati, ma in generale i principali effetti collaterali possibili sono i seguenti:
  - stanchezza
  - nausea e vomito
  - sapore cattivo o alterazione del gusto
  - perdita di appetito
  - infiammazione della bocca (stomatite), con dolore e possibili ulcere
  - infiammazione della gola e dell'esofago, con dolore alla deglutizione
  - disturbi intestinali come diarrea o stitichezza
  - infiammazione delle congiuntive
  - infiammazione della vescica con bruciore ad urinare
  - diminuzione del numero di globuli bianchi (leucopenia), le cellule del sangue che difendono dalle infezioni, quindi maggiore suscettibilità alle infezioni (febbre, talvolta con brividi, e disturbi legati alla sede dell'infezione, come tosse, mal di gola, bruciore a urinare, ecc)
  - diminuzione del numero di globuli rossi (anemia) che provoca stanchezza e talvolta difficoltà di respiro

- diminuzione del numero di piastrine (piastrinopenia), le cellule del sangue che proteggono da sanguinamenti, con possibili sanguinamenti dalle mucose (es. dal naso, epistassi) o a livello della pelle con formazione di piccole macchie rossastre (petecchie) o di lividi (ecchimosi)
- caduta di capelli e peli e talvolta di ciglia e sopracciglia
- alterazioni della pelle
- disturbi alle unghie che possono diventare secche, scheggiate o striate
- neuropatia periferica, un disturbo dei nervi periferici che si manifesta con alterazioni della sensibilità, formicolii, sensazione di punture di spilli soprattutto alle mani e ai piedi (in genere regredisce al termine delle cure, ma solo dopo diversi mesi)
- riduzione dell'udito
- alterazione della funzionalità ovarica con blocco transitorio o permanente dei cicli mestruali nella donna, possibile sterilità nell'uomo.

Inoltre, assai raramente, possono verificarsi alcune tossicità più severe, come:
- riduzione della funzionalità del cuore
- alterazioni della funzionalità del fegato
- alterazioni della funzionalità dei reni
- polmoniti di tipo non infettivo
- formazione di trombi, ossia coaguli di sangue all'interno di una vena o un'arteria.

La maggior parte dei disturbi elencati regredisce una volta completato il trattamento; tuttavia alcuni farmaci non possono essere somministrati oltre un certo dosaggio complessivo per non incorrere nel rischio di riduzioni irreversibili della funzionalità di alcuni organi. L'oncologo che l'ha in cura le spiegherà in dettaglio i possibili effetti collaterali del trattamento prescelto e attuerà tutte le misure atte a contenere tali effetti e ad evitare problemi a lungo termine.

- un trattamento ormonale, consistente, come visto sopra, in un farmaco della categoria degli inibitori dell'aromatasi (anastrozolo, letrozolo, o exemestane) oppure il fulvestrant, a cui andrà associato un LHRH agonista nelle donne in pre-menopausa e negli uomini. I principali possibili effetti collaterali degli inibitori dell'aromatasi e del fulvestrant, da soli o associati a LHRH analoghi, sono i seguenti:
  - vampate di calore e sudorazione abbondante
  - dolori articolari e osteo-muscolari
  - riduzione della densità ossea (osteopenia / osteoporosi)
  - aumento di colesterolo e trigliceridi nel sangue
  - sbalzi di umore
  - senso di stanchezza e affaticamento
  - difficoltà di memoria
  - aumento di peso
  - secchezza vaginale
  - calo del desiderio sessuale

- capogiri
- cefalea
- cataratta, irritazioni agli occhi, visione offuscata
- aumento degli enzimi epatici
- caduta capelli
- alterazioni della pelle
- prurito
- nausea e vomito
- diarrea
- abbassamento di globuli bianchi, globuli rossi o piastrine
- reazioni locali nella sede di iniezione intramuscolare per il fulvestrant
- reazioni allergiche

Inoltre, con le terapie ormonali si è riscontrato:

- un lieve aumento del rischio di disturbi della circolazione venosa o arteriosa (tromboflebite, trombosi venosa profonda, embolia polmonare, trombosi arteriosa, infarto miocardico o cerebrale), eventi che rimangono comunque molto rari.

- **Un CDK4/6 inibitore**: palbociclib, ribociclib o abemaciclib, i cui possibili principali effetti collaterali sono:
- infezioni
- abbassamento dei neutrofili (una categoria di globuli bianchi)(neutropenia)
- leucopenia
- anemia
- trombocitopenia
- neutropenia febbrile
- riduzione dell'appetito
- alterazioni del gusto
- visione annebbiata
- lacrimazione aumentata
- occhio secco
- epistassi
- stomatite
- nausea
- diarrea
- vomito
- eruzione cutanea
- alopecia
- cute secca
- affaticamento
- astenia
- piressia

- aumento di transaminasi
- allungamento dell'intervallo QT (all'elettrocardiogramma) con ribociclib (potenziale rischio di aritmie cardiache per allungamenti molto pronunciati, che comportano di dover sospendere il farmaco o ridurne le dosi)

Modalità di Assunzione dei farmaci

La somministrazione di molti chemioterapici avviene per via endovenosa ed è effettuata in ospedale. L'oncologo che la segue le spiegherà le modalità di somministrazione dei vari farmaci, nonché eventuali terapie da assumere a domicilio per contrastare gli effetti collaterali dei farmaci. Alcuni dei chemioterapici, tutti i CDK4/6 inibitori e gli inibitori dell'aromatasi vanno assunti per bocca e sarà il suo oncologo a spiegarle esattamente quando, in quali dosi e come assumere questi farmaci a domicilio e a prescrivere eventuali altri farmaci da assumere per contrastare gli effetti collaterali dei chemioterapici. Il fulvestrant viene somministrato tramite iniezione intramuscolare, che eseguirà secondo le istruzioni che il Suo oncologo le darà. Le verrà spiegato inoltre come conservare i farmaci a domicilio. **Tutti i farmaci, chemioterapici e non, devono essere conservati lontano dalla portata dei bambini**.

Quanto durerà lo studio?

Per l'esecuzione delle visite e degli accertamenti previsti dallo studio dovrà recarsi in ospedale un numero di volte variabile in base al tipo di terapia utilizzato (in genere ogni 3-4 settimane, per alcuni schemi di chemioterapia una volta alla settimana), per tutto il periodo di trattamento previsto dallo studio (ossia fino a interruzione del trattamento per progressione di malattia, tossicità, o per sua decisione o per decisione del suo oncologo).

Il medico responsabile della ricerca potrà decidere di interrompere il trattamento in studio nel caso in cui si manifestino dei problemi importanti per la Sua salute che non Le consentano di continuare la partecipazione allo studio.

Talvolta, durante uno studio clinico, si rendono disponibili nuove informazioni sul trattamento oggetto della ricerca che possono modificare il rapporto rischio/beneficio di una terapia. Se ciò dovesse accadere, Le forniremo il prima possibile le nuove informazioni che potrebbero influire sulla Sua volontà di proseguire lo studio.

Lei è comunque libero/a di interrompere la partecipazione alla ricerca quando lo ritenga opportuno, senza dover fornire alcuna spiegazione; La invitiamo a informare tempestivamente il responsabile della ricerca, qualora ciò si verifichi, ma questo non influenzerà in alcun modo la qualità delle cure mediche di cui potrebbe aver bisogno in futuro.

Interazioni con farmaci, alimenti e sostanze chimiche (compresi alcool e stupefacenti)

Poiché alcuni medicinali/alimenti/sostanze chimiche (alcool e stupefacenti) potrebbero interferire con i farmaci in studio, con potenziali rischi per la salute, Le chiediamo di rivedere con il medico responsabile dello studio, tutti i farmaci che Lei assume regolarmente o occasionalmente, compresi i prodotti da banco, i preparati di erboristeria e gli integratori dietetici.

Il medico responsabile verificherà la compatibilità di quanto Lei sta assumendo con i farmaci in studio e potrà suggerirLe dei cambiamenti. Qualora Lei non accettasse di interrompere l'assunzione di farmaci o sostanze incompatibili con i farmaci in studio, sarebbe esclusa/o dalla ricerca.

Non sono state segnalate interazioni significative tra gli inibitori dell'aromatasi (sia steroidei che non-steroidei) o il fulvestrant ed i farmaci più comunemente utilizzati, ma deve comunque essere usata cautela nell'assunzione concomitante di farmaci come rifampicina, fenitoina, carbamazepina e di preparati a base di erbe contenenti hypericum perforatum (Erba di San Giovanni) che potrebbe teoricamente ridurre l'efficacia degli inibitori dell'aromatasi. Infine, gli inibitori dell'aromatasi non devono essere assunti insieme a tamoxifen o a medicinali contenenti estrogeni, poiché questi annullerebbero la loro azione farmacologica.

I CDK4/6 inibitori presentano diverse possibili interazioni con altri farmaci:

Effetti di altri medicinali sulla farmacocinetica dei CDK4/6 inibitori:

- Effetto degli inibitori del CYP3A
  - La somministrazione concomitante di itraconazolo e CDK4/6 inibitori aumenta le concentrazioni di CDK4/6 inibitore nel sangue; deve pertanto essere evitato l'uso concomitante di forti inibitori del CYP3A compresi, ma non limitatamente a: claritromicina, indinavir, itraconazolo, ketoconazolo, lopinavir/ritonavir, nefazodone, nelfinavir, posaconazolo, saquinavir, telaprevir, telitromicina, voriconazolo e pompelmo o succo di pompelmo.
  - Non è necessario alcun aggiustamento della dose per inibitori del CYP3A lievi e moderati.
- Effetto degli induttori del CYP3A
  - La somministrazione concomitante di rifampicina e CDK4/6 inibitori riduce le concentrazioni di CDK4/6 inibitore nel sangue; deve pertanto essere evitato l'uso concomitante di forti induttori del CYP3A compresi, ma non limitatamente a: carbamazepina, enzalutamide, fenitoina, rifampicina ed erba di San Giovanni.
  - La somministrazione di induttori moderati del CYP3A è invece consentita.
- Effetti dei CDK4/6 inibitori sulla farmacocinetica di altri medicinali
  - Può essere necessario ridurre la dose di substrati del CYP3A sensibili con un ristretto indice terapeutico (ad esempio alfentanil, ciclosporina, diidroergotamina, ergotamina, everolimus, fentanil, pimozide, chinidina, sirolimus e tacrolimus) quando co-somministrati con CDK4/6 inibitori, poiché questi possono aumentare la loro concentrazione nel sangue.
- Palbociclib va assunto a stomaco pieno.

Le chiediamo inoltre di avvisare preventivamente il Medico responsabile dello studio riguardo alla Sua intenzione di assumere un qualsiasi nuovo farmaco/sostanza.

Lei è obbligato/a a partecipare a questa ricerca?
La Sua decisione di partecipare a questa ricerca è completamente **libera e volontaria**.

Lei è libero/a di decidere di ritirarsi dalla ricerca in qualsiasi momento e senza dare alcuna spiegazione, senza alcuna penalità o perdita di benefici.

I dati raccolti fino al momento del ritiro potranno essere comunque utilizzati e non saranno raccolti ulteriori dati.

Contraccezione, Gravidanza e Allattamento
Uomini e donne sessualmente attivi devono usare efficaci metodi di contraccezione durante lo studio e per i 6 mesi successivi all'ultima assunzione dell'ultimo trattamento sperimentale.

Poiché chemioterapici e farmaci ormonali possono arrecare danni al feto o, in caso di allattamento, al bambino, se Lei è una paziente in età fertile **deve impegnarsi a non iniziare una gravidanza** per tutta la durata dello studio e per i 6 mesi successivi all'ultima assunzione dell'ultimo trattamento sperimentale, e a **non allattare per il medesimo periodo**.

Poiché i farmaci LHRH analoghi, pur bloccando il ciclo mestruale, non sono sufficientemente sicuri come anticoncezionali, è necessario utilizzare metodi contraccettivi più efficaci (per es. astinenza completa, se ciò è in linea con le Sue preferenze; metodi combinati di barriera e sistemi intrauterini). La pillola anticoncezionale (estro-progestinica) risulta invece controindicata in pazienti con tumore mammario.

Si consiglia quindi di intraprendere metodi contraccettivi altamente efficaci come:
- contraccezione di barriera doppia
- spirale contraccettiva (IUD)
- sterilizzazione tubarica
- vasectomia del partner
- astinenza

Per qualsiasi domanda in merito ai metodi contraccettivi, La preghiamo di rivolgersi al Medico Responsabile dello studio.

Se Lei è una donna in età fertile, all'inizio dello studio verrà sottoposta a un test di gravidanza. Nel caso si verificasse una gravidanza o il sospetto di gravidanza, dovrà informarne immediatamente il Medico Responsabile dello studio.

Quali rischi correrà se parteciperà a questa ricerca?
I farmaci utilizzati nello studio sono in commercio per lo più da anni in Italia ed ampiamente utilizzati. Se ne conoscono pertanto dettagliatamente gli effetti collaterali, anche quelli molto rari. Di più recente introduzione, ma già ampiamente sperimentati e utilizzati nella pratica clinica, sono i CDK4/6 inibitori.

Lei deve informare della Sua partecipazione allo studio il personale sanitario che La prende in cura, specialmente se sta per sottoporsi ad un trattamento chirurgico, dentario, o a un qualsiasi altro trattamento.

Le viene consegnata una lettera destinata al Suo medico curante. La lettera contiene tutte le informazioni relative alla Sua partecipazione allo studio, in particolare l'indicazione degli effetti delle terapie che riceverà durante la sperimentazione, dei farmaci che non deve assumere e dei trattamenti medici ai quali non deve sottoporsi, in quanto incompatibili o sconsigliati.

La invitiamo, nel Suo interesse e a tutela della Sua salute, a: 1) consegnare la lettera al Suo medico curante; 2) informare i medici che La seguono nella sperimentazione di ogni farmaco o prodotto omeopatico o fitoterapico che intendesse assumere e di ogni trattamento medico al quale intendesse sottoporsi.

Qualora Lei omettesse di fornire tali indispensabili informazioni, né il Suo medico curante, né i medici che La seguono nella sperimentazione potranno essere considerati responsabili per i danni derivanti dalla incompatibilità tra la sperimentazione e ogni diverso trattamento medico. La avvertiamo inoltre che tali danni potrebbero non essere coperti dall'assicurazione stipulata al momento della Sua partecipazione allo studio.

Durante lo studio non deve effettuare donazioni di sangue e/o di emocomponenti.

Se, in qualsiasi momento, Lei dovesse sperimentare un effetto collaterale, bisognerà informarne al più presto il Medico Responsabile della ricerca senza aspettare la prossima visita programmata, al fine di poter prendere, quando necessario, dei provvedimenti.

Durante la partecipazione a questa ricerca Lei potrebbe essere sottoposto/a ai seguenti esami/indagini strumentali che presentano questi rischi:
-   TAC: l'esame comporta l'esposizione a radiazioni ionizzanti, che sono potenzialmente dannose per la salute, perché potrebbero nel tempo causare i tumori; tuttavia questa evenienza è molto rara, e passa del tutto in secondo piano in pazienti già affetti da tumore, nei quali è molto più importante valutare l'efficacia e quindi la correttezza della terapia del tumore presente, eseguendo periodiche valutazioni dell'andamento della malattia con esami diagnostici come la TAC, piuttosto che preoccuparsi di un ipotetico secondo tumore che potrebbe verificarsi anni dopo.
-   Scintigrafia ossea e PET: tali esami prevedono la somministrazione endovenosa di sostanze radioattive, che emettono radiazioni per circa 24 ore; la dose di radiazioni ricevute da chi si sottopone all'esame è equivalente circa a quella che si assume quando si esegue una TAC; una piccola quota di radiazioni può però raggiungere anche persone vicine alla/al paziente, per cui è raccomandato che ella/egli eviti il contatto con donne in gravidanza e bambini piccoli, fintanto che la radioattività non sia scomparsa dall'organismo (circa 24 ore).
-   Risonanza magnetica: questa non espone a radiazioni ionizzanti; non possono sottoporsi a risonanza magnetica i portatori di pacemaker cardiaco o di neurostimolatori perché il campo magnetico o le onde prodotte dall'apparecchiatura potrebbero alterarne il funzionamento; più in generali non possono sottoporsi a risonanza magnetica persone portatrici di apparecchiature metalliche (esempio pacemaker, protesi, chiodi e viti applicate in ortopedia, clips vascolari) o schegge metalliche (in seguito a incidenti o ad attività lavorative) nel corpo, poiché queste potrebbero muoversi in seguito all'esposizione al forte campo magnetico e provocare danni ai tessuti di vario genere. La maggior parte delle apparecchiature mediche impiantate attualmente è compatibile con la risonanza magnetica, ma quelle più vecchie possono non esserlo: è sempre necessario

quindi verificare la compatibilità discutendone con il medico e/o consultando il materiale informativo delle apparecchiature.
- Ecografia: non comporta rischi per la salute.

I farmaci in studio Le apporteranno dei benefici?
I risultati di ricerche già condotte ci fanno pensare che i trattamenti in studio possano migliorare la Sua salute, però non ci sono garanzie assolute in tal senso, quindi non possiamo garantirLe né prometterLe effetti benefici certi.
Tuttavia Lei potrà giovarsi di un attento monitoraggio delle Sue condizioni fisiche, e avrà la possibilità di dare il Suo contributo ad una ricerca scientifica che in futuro potrebbe aiutare persone che hanno la Sua stessa malattia.

Per la sua partecipazione allo studio è previsto un compenso?
Non è previsto alcun compenso per la Sua partecipazione allo studio. Allo stesso tempo Lei non dovrà pagare nulla per le visite, gli esami ed i farmaci previsti da questo studio.

Chi ha esaminato lo studio?
Questo studio è stato approvato dal Comitato Etico della Romagna (CEROM), ossia il comitato che garantisce la tutela dei diritti dei soggetti umani presso IRST IRCCS ed Area Vasta Romagna, promotore e centro coordinatore dello studio.
Lo studio è stato inoltre approvato dal Comitato Etico _____ a cui fa riferimento il Medico Responsabile dello studio presso il Centro Partecipante di _____.

La ricerca ha una copertura assicurativa?
Il Promotore che ha commissionato lo studio ha stipulato apposita polizza di Responsabilità Civile verso Terzi con la società Lloyd's che prevede il risarcimento dei danni causati durante la sperimentazione in conformità al protocollo.
La polizza assicurativa garantisce la copertura dei danni da responsabilità civile derivante dalla sperimentazione stessa fino a € 1.000.000 per paziente, non copre il valore eccedente il massimale, ed è operante esclusivamente per i danni manifestati entro 24 mesi dalla conclusione della sperimentazione e per richieste di risarcimento presentate entro e non oltre 36 mesi dalla conclusione della sperimentazione stessa. Tale limitazione non inficia comunque il diritto del soggetto danneggiato ad ottenere il risarcimento da parte del responsabile dell'eventuale danno. Sono esclusi dalla copertura della polizza assicurativa:
- i danni causati da sperimentazioni non regolarmente autorizzate e/o svolte in maniera difforme da quanto autorizzato
- i danni che non siano in relazione causale nei termini stabiliti dalla legge 211/2003 con la sperimentazione assicurata
- i reclami dovuti al fatto che la formulazione farmaceutica soggetta a sperimentazione non realizza gli scopi curativi previsti

- i danni congeniti o malformazioni, provocate in donne incinte partecipanti alla sperimentazione
- i danni genetici infermità genetiche e/o ereditarie
- i danni nucleari di qualsiasi tipo
- i reclami dovuti ad immunodeficienza acquisita da HIV o ad errata diagnosi di tale sindrome
-

Si specifica che per tutte le procedure secondo pratica clinica si fa riferimento alla copertura assicurativa aziendale del Centro Partecipante presso cui è in cura.

Si precisa che per tutta la durata dello studio saranno operative idonee coperture assicurative.

Per qualsiasi domanda riguardante la garanzia di risarcimento e le cure mediche e per qualunque disturbo o danno Lei dovesse ritenere correlato alla sperimentazione La preghiamo di contattare:

Nome e Cognome: _____ Tel nr.: _____

Aspetti etici

La ricerca cui Le è stato proposto di partecipare, incluso il presente modulo informativo, è stata esaminata ed approvata dal Comitato Etico di questa struttura. Lo studio è stato progettato e verrà condotto in conformità agli standard etici internazionali e nazionali sulla ricerca biomedica con esseri umani: in particolare, alle revisioni correnti della dichiarazione di Helsinki dell'Associazione Medica Mondiale sui "Principi etici per la ricerca medica che coinvolge soggetti umani" e delle Norme di Buona Pratica Clinica (ICH/GCP) della Unione Europea sulla sperimentazione dei medicinali laddove applicabili; alla Convenzione del Consiglio d'Europa per la protezione dei diritti dell'uomo e della dignità dell'essere umano nell'applicazione della biologia e della medicina (Convenzione di Oviedo del 04/04/1997); ai contenuti dei codici italiani di deontologia delle professioni sanitarie e della specifica normativa nazionale vigente in tema di studi clinici. Lei può segnalare qualsiasi fatto ritenga opportuno evidenziare, relativamente allo studio che La riguarda, al Comitato Etico di questa struttura. In nessun caso l'approvazione del Comitato Etico deve essere da Lei considerata come un incoraggiamento a partecipare a questo studio.

Come sarà protetta la sua riservatezza?

Il Suo consenso alla partecipazione a questa ricerca significa che Lei autorizza l'uso dei dati personali e sensibili contenuti nella Sua cartella clinica, in maniera anonima per esclusivi motivi di ricerca. Questi dati potranno anche essere confrontati a scopo statistico con dati analoghi provenienti da altre fonti epidemiologiche o cliniche. Tutte le informazioni (personali, cliniche) raccolte durante questa ricerca sono confidenziali e verranno trattate nel rispetto della normativa vigente (Reg UE 679/2016 – Regolamento Generale sulla Protezione dei Dati - "GDPR").

Alla fine della ricerca i risultati potranno essere pubblicati ma la sua identità resterà anonima.

La informiamo inoltre che la documentazione clinica originale che la riguarda potrà essere visionata dal Promotore dello studio o da suoi rappresentanti, dal Comitato Etico o dalle

Autorità regolatorie di governo, quali ad esempio il Ministero della Salute italiano, la Food and Drug Administration (Stati Uniti), per verificare che le informazioni riportate sui documenti dello studio siano corrette e vere.

Lei può decidere di NON dare il Suo consenso all'utilizzo dei suoi dati, ma in tal caso NON potrà partecipare alla ricerca. Può anche decidere di ritirare il Suo consenso a partecipare in qualsiasi momento, tuttavia le informazioni raccolte sino al Suo ritiro potranno comunque essere utilizzate come dati dello studio.

Lei ha il diritto di vedere le informazioni personali che La riguardano, ad es. nome, indirizzo, e ha il diritto di correggere tali dati se necessario.

Per maggiori informazioni sulla protezione dei dati consulti il documento "Informativa e manifestazione del consenso al trattamento dei dati personali"

Cosa significa dare il consenso informato?

Se acconsente a partecipare alla ricerca, dovrà firmare l'allegato modulo di consenso. La firma di questo modulo non Le toglierà in alcun modo i Suoi diritti; essa viene richiesta soltanto al fine di garantire che Lei sia stata/o informata/o in modo completo sullo studio, che ne abbia capito lo scopo ed il Suo coinvolgimento.

Prima di firmare verifichi, per cortesia, se Le è tutto chiaro riguardo allo studio e a quello che dovrà fare; se Le restano dei dubbi non abbia timore di richiedere ulteriori spiegazioni.

Se ha qualsiasi domanda, incluse quelle inerenti questo studio o riguardanti i Suoi diritti, o se crede di essere stata/o danneggiato/a in qualunque modo partecipando a questo studio, La preghiamo di farlo presente al Medico responsabile della ricerca ora oppure nel corso dello studio, contattandolo al numero telefonico qui di seguito riportato. Il Medico responsabile della ricerca ed il personale che lo assiste nella conduzione della ricerca sarà lieto di rispondere a qualsiasi Sua domanda. Nessuna pressione verrà fatta su di Lei affinché partecipi a questo studio.

Se è d'accordo, il Medico responsabile della ricerca informerà il Suo Medico curante della Sua partecipazione a questa ricerca.

Lei ha il diritto in ogni momento di fare domande sulla ricerca e di richiedere comunicazioni ed informazioni circa lo studio al: Dr./Prof. _____ presso l'Ospedale/Reparto/N. tel. _____.

Libero arbitrio – Diritti dell'interessato – Revoca del consenso

La Sua collaborazione è libera e volontaria. Pertanto Lei è libera/o di revocare, in ogni momento il Suo consenso. Le richieste di esercizio dei diritti previsti dal Codice a favore dell'interessato (ad esempio, avere comunicazione dei dati trattati che La riguardano, conoscerne l'origine, la logica, le finalità e le modalità su cui si basa il trattamento; ottenerne l'aggiornamento, la rettifica o l'integrazione) possono essere rivolte al medico di riferimento che le ha proposto di partecipare a questo studio.

Si sottolinea che il protocollo sperimentale è stato redatto in conformità alle Norme di Buona Pratica Clinica e nel rispetto dei principi etici stabiliti nelle dichiarazioni internazionali.

La ringraziamo anticipatamente del Suo aiuto per questa ricerca.

MODULO DI CONSENSO INFORMATO

Sigla di identificazione dello studio: IRST 174.19
Titolo dello studio: Studio clinico con randomizzazione sequenziale adattativa a gruppi di confronto tra chemioterapia + endocrinoterapia verso inibitori delle chinasi ciclina-dipendenti 4 e 6 (CDK4/6) + endocrinoterapia nel carcinoma mammario avanzato a recettori ormonali positivi e HER2-negativo.
Data e Versione N°: 09/11/2018 – Emendamento 1.0

Sperimentatore Principale: _____

Promotore dello studio: Istituto Scientifico Romagnolo per lo Studio e la Cura dei
                                                  Tumori (IRST) IRCCS

L'INFORMAZIONE AL PAZIENTE IN VIRTÙ DELLA PROPEDEUTICITÀ DI TALE FASE DOVRÀ ESSERE FORNITA IN UN MOMENTO PRECEDENTE E FORMALMENTE DISTINTO DAL RECEPIMENTO DEL CONSENSO.

Io   sottoscritta/o   _____,
firmando il presente documento:

1. Confermo di aver ricevuto dal Dr._____
   informazioni scritte e verbali sullo studio in oggetto e sulle previsioni di rischi e benefici connessi alla sperimentazione, e di aver ricevuto copia del foglio informativo e del presente modulo di consenso firmato e datato.
2. Confermo di avere avuto il tempo necessario per porre tutte le domande per me importanti relative allo studio. Sono soddisfatta/o di tutte le risposte che mi sono state date.
3. Sono consapevole che la mia partecipazione a questo studio è volontaria e che sono libera/o di ritirami dalla ricerca in ogni momento, senza darne ragione e senza incorrere in problemi o svantaggi per la mia assistenza medica futura.
4. Sono consapevole che il Promotore della ricerca ha stipulato apposita polizza di Responsabilità Civile che prevede il risarcimento di danni o lesioni causate dall'esecuzione della ricerca.
5. Sono consapevole della necessità, diretta a garantire la migliore tutela della mia salute, di informare il mio medico curante della sperimentazione alla quale accetto di partecipare, consegnandogli la lettera a lui indirizzata; nel caso decida di non informarlo o ometta di farlo, né il mio medico curante né i medici che mi seguono nella sperimentazione saranno considerati responsabili per i danni che possano derivare dall'incompatibilità tra la sperimentazione e qualunque altro diverso trattamento medico o farmaco o prodotto omeopatico o fitoterapico io abbia assunto. Inoltre sono

consapevole che tali danni potrebbero non essere coperti dall'assicurazione stipulata al momento della mia decisione di partecipare allo studio.

- **autorizzo**
- **non autorizzo**

a fornire al mio Medico di Medicina Generale notizie sulla mia partecipazione a questo studio clinico.

6. · **desidero**
   · **non desidero**

ricevere informazioni da parte del Medico Responsabile dello studio sui risultati della ricerca.

7. · **desidero**
   · **non desidero**

ricevere informazioni da parte del Medico Responsabile dello studio sugli eventuali risultati accidentali che dovessero derivare dalla ricerca.

8. Sono consapevole del fatto che la mia partecipazione allo studio comporta la raccolta e l'elaborazione dei miei dati personali, compresi quelli sensibili. Tutti i dati verranno comunque raccolti in forma anonima, ai sensi del Regolamento (UE) 2016/679 in materia di protezione dei dati personali, e solo per quanto risulterà necessario ai fini dello studio sopra citato.

9. Sono stata/o informata/o dell'approvazione dello studio da parte del Comitato Etico Indipendente locale, a tutela della correttezza della sperimentazione e dei diritti del malato.

Il Dr _____ mi ha consegnato copia firmata e datata di questo modulo di consenso informato e del foglio informativo.

Acconsento a partecipare a questo studio.

Nome e Cognome del Soggetto (in stampatello):

_____

Data_____/_____/_____ Firma del Soggetto _____
(da apporre da parte del Soggetto)

Nome e Cognome del Rappresentante Legale* (in stampatello):

_____

Data_____/_____/_____

Firma del Rappresentante Legale_____

*In caso sia designato quale rappresentante legale un amministratore di sostegno, il medico sperimentatore avrà cura di verificare che l'ordinanza di affidamento da parte del giudice tutelare comprenda anche la tutela della salute dell'amministrato

Nome e Cognome del Testimone Imparziale* (in stampatello): **_____**

Data_____/_____/_____ Firma del Testimone Imparziale _____

Nome e Cognome del Testimone Imparziale* (in stampatello): **_____**

Data_____/_____/_____ Firma del Testimone Imparziale _____

* una persona che sia indipendente dallo studio, che non possa essere influenzata in alcun modo dalle persone coinvolte nello studio, che partecipi alla procedura di informazione del paziente se il paziente non è in condizione di leggere e che legga il consenso informato e ogni altra informazione scritta fornita al paziente partecipante

DA COMPLETARE DA PARTE DEL MEDICO DELLO STUDIO CHE HA OTTENUTO IL CONSENSO

Confermo di aver fornito al paziente esaurienti spiegazioni circa la natura, lo scopo, la durata e i possibili rischi connessi con lo studio in argomento e di avergli consegnato una copia del foglio informativo ed una copia datata e firmata del modulo di consenso.

Nome e Cognome del Medico dello studio che ha ottenuto il consenso (in stampatello)

_____

Data_____/_____/_____

Firma del Medico che ha ottenuto il consenso _____

Appendix 4

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI

**Group sequential response adaptive randomized clinical trial of chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer**

Short title / Acronym: CDK4/6-inhibitor or chemotherapy, in combination with ENDOcrine therapy, for advanced breast cancer / KENDO

Protocol Code: IRST174.19

IRST-Identifier Code: L2P1388

Eudract number: 2016-004107-31

Date and Version No: 09/11/2018 – Amendment 1.0

Chief Investigator: Andrea Rocca
Head of breast cancer unit
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Confidentiality Statement

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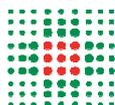
PROTOCOL SIGNATURE PAGE

Group sequential response adaptive randomized clinical trial of chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer

EudraCT number: 2016-004107-31

The undersigned agree and confirm that:

The following protocol has been agreed and accepted and the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the ICH GCP guidelines, the European Directive, 2001/20/CE, Italian Decree 211/2003, Promoter SOP's and other regulatory requirements as amended.

The confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Promoter.

The findings of the study will be made publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and any discrepancies from the study as planned in this protocol will be explained.

Andrea Rocca
Chief Investigator   Signature   Date

Alessandro Vagheggini
Trial Statistician   Signature   Date

By signing this document I am confirming that I have read the protocol for the above study and I agree to conduct the study in compliance with the protocol and ICH GCP

Principal Investigator   Signature   Date

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EMILIA-ROMAGNA**
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Istituto di Ricovero e Cura a Carattere Scientifico

ISTITUTO
SCIENTIFICO
ROMAGNOLO
PER LO STUDIO E LA CURA
DEI TUMORI

Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0

SUMMARY

Title	Group sequential response adaptive randomized clinical trial of concomitant chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer
Short Title/ Acronym	CDK4/6-inhibitor or chemotherapy, in combination with ENDOcrine therapy, for advanced breast cancer / KENDO
Protocol Code	IRST174.19 Identifier Code: L2P1388
Phase	Phase 2
Study Design	Prospective, open label, multicenter, group sequential response adaptive randomized phase 2 study, comparing two treatments for locally advanced or metastatic luminal breast cancer: - Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (aromatase inhibitor [AI] or fulvestrant) - Arm B: chemotherapy plus endocrine therapy (AI or fulvestrant, administered either concomitantly from the beginning of chemotherapy or sequentially after 4-6 months of chemotherapy) Treatments will continue until disease progression or toxicity or patient refusal. Cross-over to the other treatment arm is encouraged (although not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy.
Back ground and Rationale for study	Although metastatic luminal breast cancer is most often treated with endocrine agents as first-line therapy, chemotherapy may be useful in specific conditions, and is eventually administered to most patients after the onset of endocrine resistance. The choice and sequence of treatments for metastatic luminal breast cancer depends on responsiveness to previous (e.g. adjuvant) therapies and on biological and clinical features. Chemotherapy is used in earlier lines of treatment in presence of signs of disease aggressiveness, such as short disease-free interval, elevated Ki67 (preferably, if available, on a metastatic biopsy), low expression of hormone receptors (HRs),

extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms.

Several new targeted agents are being developed in breast cancer, and some of them have been shown to improve the efficacy of endocrine therapy when given in combination with endocrine agents, and to overcome or delay the development of endocrine-resistance. The cyclin-dependent Kinase 4 and 6 (CDK4/6) inhibitors (palbociclib, ribociclib, abemaciclib) significantly improve progression-free survival when given in combination with an aromatase inhibitor (AI) as first-line treatment of endocrine-sensitive metastatic breast cancer, or when given in combination with fulvestrant in patients with HR-positive metastatic breast cancer after progression to an aromatase inhibitor. Therefore, a combination of an endocrine agent (AI or fulvestrant) and a CDK4/6 inhibitor has become the preferred first-line treatment in patients with HR-positive metastatic breast cancer, except in cases of very indolent disease or poor performance status, when endocrine therapy alone is still preferred, or in cases of very aggressive disease which may still be treated with first-line chemotherapy. The combinations of CDK4/6 inhibitors and endocrine therapy could potentially replace chemotherapy alone in some cases of aggressive disease, and studies comparing the two strategies are ongoing.

Preclinical studies show a synergism between chemotherapy and some endocrine agents such as AIs and fulvestrant. Therefore, a combination of chemotherapy and endocrine therapy could be a further, potentially very active, treatment strategy in metastatic luminal breast cancer.

The choice between a chemotherapy-based and a CDK4/6 inhibitor-based treatment remains particularly controversial in patients with doubtful endocrine sensitivity, e.g. due to a low expression of estrogen receptors (ERs), or of primary endocrine resistance, indicated by a short disease-free interval.

We plan to conduct a phase II group sequential response adaptive randomized clinical trial comparing the combination of chemotherapy plus endocrine therapy with CDK4/6 inhibitors plus endocrine therapy in patients with advanced HR-positive, HER2-negative breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement

or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option.

The main aim is to assess if a combination of chemotherapy and endocrine therapy is superior to CDK4/6 inhibitors plus endocrine therapy in terms of progression-free survival. Secondary aims are comparing the two treatment arms in terms of disease control rate (objective response or stable disease after 3 months of treatment), objective response rate, overall survival, toxicity and patient reported outcomes. In a biological correlative study, the expression and mutational/ Copy Number Variation (CNV) profiles of genes involved in pathways subtending cell cycle progression (cyclin D – CDK4/6 – Rb), as targets of CDK4/6 inhibitors, and response to chemotherapy (p53, p38, DNA damage response) will be assessed on tumor specimens. The same gene mutational profiles and CNVs will be assessed on Circulating Tumor Cells (CTC) with analysis at single cell level. Also, on a subgroup of patients enrolled at Meldola, Forlì and Cesena, the expression profiles of genes involved in the pathways of interest (cyclin D – CDK4/6 – Rb, p53, p38, DNA damage response) will be assessed on CTCs at single cell level.

Timelines	Estimated duration for the amended protocol: 39 months Study start (FPFV): 12/2018 Recruitment end (LPFV): 10/2020 Follow-up period end date (LPLV): 02/2022	
Study Centers	Multi-center study	
Objectives and outcome measures	Primary	To compare the efficacy of concomitant CDK4/6 inhibitor plus endocrine therapy versus chemotherapy plus endocrine therapy (administered either concomitantly from the beginning or sequentially) in terms of progression-free survival (PFS).
	Secondary	To compare between treatment arms: • quality of life (EORTC QLQ-C30 and QLQ-BR23) • toxicity (CTCAE version ~~45.0~~3) • time to treatment failure • best response rate • duration of response • clinical benefit rate • overall survival (OS) • PFS and clinical benefit with the subsequent line of treatment after cross-over: CDK4/6 inhibitors plus endocrine therapy in patients

		treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy
		• correlative biomarkers of response to CDK4/6 inhibitors and chemotherapy:
		    – tissue markers (on the primary tumor and / or metastatic tissue)
		    – circulating markers (e.g. CTCs, ctDNA)
		To compare, within the experimental arm, PFS with concomitant versus sequential administration of chemotherapy and endocrine therapy (exploratory endpoint)

Number of Subjects		Actual sample size will depend on the possibility of early stopping for efficacy at any of the two planned interim analyses. However, at the most an overall sample size of 150 patients is planned. Patients will be allocated according to group sequential response adaptive randomization.

Diagnosis and Main Eligibility Criteria		Patient population: postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not) with HR-positive, HER2-negative, locally advanced or metastatic breast cancer, with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.
		<u>Inclusion criteria:</u>
		• Histological diagnosis of HR-positive (ER ≥10% of tumor cells), HER2-negative (according to ASCO guidelines 2018) breast cancer, determined by local laboratory on most recent available tumor tissue.
		• Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as "advanced breast cancer (ABC)").
		• At least one of the following signs of disease aggressiveness:
		    o The main criteria are a low expression of ER (10% ≤ ER < 50%) and/or a relapse while on the first 2 years of adjuvant endocrine therapy or disease progression (PD) within the first 6 months of first-line endocrine therapy for ABC
		    o Other tumor characteristics of aggressiveness that make the patient potentially candidate to chemotherapy, according to the guidelines of the Italian Association of Medical Oncology [AIOM guidelines 2017], such as: elevated Ki67 (preferably documented, if available, on a metastatic biopsy), low

expression of hormone receptors (e.g. progesterone receptor <20%), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms; these patients are eligible if chemotherapy is considered a suitable option by the treating physician.

- Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not).
- Measurable disease according to RECIST 1.1 criteria, or not measurable but evaluable disease.
- Any prior adjuvant chemotherapy or endocrine therapy
- No prior chemotherapy for advanced disease.
- Up to one prior line of endocrine therapy for ABC.
- Age ≥ 18 years.
- Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).
- Adequate organ (renal, hepatic, bone marrow, cardiac) functions.
- Female participants of child bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.
- Participant is willing and able to give informed consent for participation in the study.

Exclusion criteria:

- Any prior chemotherapy or CDK4/6 inhibitor for advanced breast cancer
- More than 1 prior line of endocrine therapy for ABC.
- Patients who have not recovered from adverse events due to prior therapies to grade ≤1 (excluding alopecia).
- Active central nervous system metastases.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to the drugs used in the study.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

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	• Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.
Study Product, Dose, Route, Regimen and duration of administration	Chemotherapy regimen: at the discretion of the treating physician (treatment of physician choice, TPC), based also on patient's features and preferences. Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard". Chemotherapy regimens will be classified as: ▪ anthracycline + taxane, ▪ taxane, ▪ anthracycline, ▪ capecitabine / fluoropyrimidines, ▪ others. Endocrine therapy: ▪ non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI ▪ non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed while on adjuvant steroidal AI ▪ steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed while on adjuvant non-steroidal AI ▪ fulvestrant in women not pretreated with fulvestrant for advanced disease CDK4/6 inhibitor: ▪ palbociclib ▪ ribociclib ▪ abemaciclib Any cytotoxic or endocrine agent to which a patient was exposed in the adjuvant setting may be administered within this study only if PD occurred at least 12 months after the end of that drug.
Reference therapy	The standard reference therapy is CDK4/6 inhibitor plus endocrine therapy.

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Statistical Methodology	The patients will be allocated according to block randomization until two events are observed in each arm, and then according to the time-to-event adaptation (Zhang and Rosenberger, 2007) of the group sequential Doubly-adaptive Biased Coin Design (DBCD) whose allocation probabilities are computed at the end of the block randomization and after around 70% and 85% of the 150 maximum patients are enrolled during a 23 month period. At these last two (i.e. after 105 and 128 patients, respectively), interim analysis on efficacy (Zhu and Hu, 2010) will be carried out allowing for early stopping. Assuming for the survival times an exponential distribution parametrized in terms of its expected value, the null hypothesis of equality of PFS versus a higher one for arm B ($H_0: \theta_A = \theta_B$; $H_1: \theta_B > \theta_A$) will be tested by means of the nonparametric log-rank test with a 10% significance level. The adoption of the Lan and DeMets (1983) α-spending function for determining the upper boundaries allows to preserve the nominal level throughout the two interim analyses. At the end of the 16-month follow up, administrative censoring is introduced. Therefore, the total study duration is 39 months.
	Simulations carried out assuming different scenarios showed good operating characteristics, especially when compared with the ones of the usual complete randomization (CR) coupled with the same test statistics on a fixed 150 patient sample size. Previous results on palbociclib and fulvestrant combination in second line (Paloma 3 Study, supplementary material of Turner NC, NEJM 2018) and the characteristics of our target population lead us to assume a median PFS of 8 and 12 months for arm A and B, respectively. Under this scenario, for a sample size of at the most 150 patients, the proposed design strategy has led to a simulated power of 0.911 compared with a 0.717 one for the CR design. Moreover, a reduced expected sample size (ESS) of 121.259 patients is observed due to early stopping for efficacy, whereas CR forces all 150 patients to be equally assigned to both arms. Furthemore, assuming a median PFS of 8 and 12 months for Arm A and B, respectively, simulations showed that group sequential DBCD allocates around 56% of the patients to Arm B.

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ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI

STUDY SCHEMA

Trial scheme

Disease:
HR-positive, HER2-negative locally advanced or metastatic **breast cancer (ABC)**, with features of aggressiveness

Treatment:

Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy

Permuted Block Randomization → **Response Adaptive Randomization** (the allocation probabilities updating process will take place after 105 and 128 patients are enrolled along with interim analysis on efficacy allowing for early stopping of the trial)

Permuted Block Randomization (until two relapses are experienced in both arms)

Treatments:
CT:
- anthracyclines + taxanes
- taxanes
- anthracyclines
- fluoropyrimidines
- others

ET:
- non-steroidal AI
- steroidal AI
- fulvestrant

CKK4/6 inhibitor:
- palbociclib
- ribociclib
- abemaciclib

Arm B: chemotherapy plus endocrine therapy

Treatments will continue until disease progression or toxicity or patient refusal

Cross-over is encouraged (although not mandatory)

ABBREVIATIONS

ABC	Advanced Breast Cancer
AE	Adverse Event
AI	Aromatase Inhibitor
ALT	Alanine aminotransferase (previously SGPT)
ANC	Absolute neutrophil count
ASAT	Aspartate aminotransferase (previously SGOT)
AR	Adverse reaction
BSA	Body surface area
CBC	Complete blood count
CC	Coordinating Centre
CI	Confidence interval
CNS	Central Nervous System
CT	Chemotherapy
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRO	Contract Research Organisation
CTscan	Computed tomography
CTC	Common toxicity criteria
CTCs	Circulating Tumor Cells
DFS	Disease Free Survival
DFI	Disease Free Interval
ECG	Electrocardiogram
ECOG-PS	Eastern Cooperative Oncology Group Performance status
eCRF	electronic Case Report Form
ET	Endocrine Therapy
FPFV	First Patient First Visit
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor

GP	General Practitioner
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IDMC	Indipendent Data Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Products
IRB	Independent Review Board
LPFV	Last Patient First Visit
LP	Last Patient
LPLV	Last Patient Last Visit
ORR	Overall Response Rate
OS	Overall survival
PD	Progressive disease
PFS	Progression Free Survival
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PR	Partial response
RECIST	Response Evaluation Criteria In Solid Tumors
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Stable disease
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TPFS	Total Progression Free Survival
TTP	Time to progression
ULN	Upper limit of normal
WBC	White blood cells
WHO	World Health Organization

TABLE OF CONTENTS

1. INTRODUCTION

This document is a protocol for a human research study. This clinical trial is to be conducted in compliance with the protocol, with the European Directive, 2001/20/CE and Italian Decree 211/2003, with the principles of ICH Good Clinical Practice, and institutional research policies and procedures.

1.1 Background

Luminal breast cancer

Studies of gene expression profiling have led to the definition of different subtypes of breast carcinoma, including the luminal A and B, HER2-enriched, basal-like and claudin-low subtypes, with important differences in prognosis and response to treatments [Brenton JD 2005].

The luminal subtypes are characterized by the expression of estrogen receptors (ER) and genes associated with ER activation, such as CCND1 (encoding cyclin D1), as well as the expression of low molecular weight "luminal" cytokeratins (CKs) like CK 8 and 18, which are usually found in the luminal layer of stratified epithelia. Multiple studies have consistently reproduced two luminal subtypes: the luminal A, with higher expression of ER and its related cluster of genes and low expression of proliferative genes, and the luminal B [Ades F 2014], with lower expression of ER and its related genes and higher expression of proliferative genes, such as CCNB1, MKI67, and MYBL2 [Hu Z 2006], as well as of growth factor receptor genes [Wirapati P 2008].

For practical purposes, intrinsic breast cancer subtypes are approximated using clinicopathological criteria [Goldhirsch A 2011, Goldhirsch A 2013], and luminal B tumors are often identified as ER-positive with high Ki67 [Cheang MC 2009], or ER-positive and HER2-positive [Goldhirsch A 2011, Goldhirsch A 2013], or ER-positive with a low expression of progesterone receptors (PgR) [Prat A 2013]. In this study, we will consider only HER2-negative luminal B tumors, defined as ER-positive (>10% of tumor cells) and at least one of the following: PgR low (≤20%) or Ki67 high (≥ 20%).

Prognosis of luminal B breast cancer is significantly worse compared to luminal A tumors and in untreated cases is more similar to that of basal-like and HER2-enriched subtypes [Hu Z 2006; Ades F 2014].

Treatments commonly used for luminal tumors include endocrine therapy, often combined with other targeted agents such as cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors, and chemotherapy. Responsiveness to these treatments varies between luminal A and luminal B tumors. Luminal B tumors are less responsive to chemotherapy compared with HER2-enriched and basal-like subtypes, but more responsive than luminal A tumors, as highlighted by studies of neoadjuvant chemotherapy [Esserman LJ 2012]. In the adjuvant setting there is evidence of advantage from newer regimens, including anthracyclines or taxanes, over older ones in patients with luminal B tumors [Hugh J 2009], but not in those with luminal A tumors. On the other hand, luminal B tumors are less endocrine-sensitive compared with luminal A tumors, as shown by the predictive value of progesterone receptor (PgR) levels [Bardou VJ 2003] and Ki-67 [Viale G 2008] for benefit from adjuvant endocrine therapies. In the adjuvant setting, benefit from chemotherapy added to endocrine therapy appears confined to patients with tumors with high recurrence score, a measure of recurrence risk based largely on levels of transcription of genes involved in cell proliferation and of ER-related genes [Albain KS 2010]. CDK4/6 inhibitors work preferentially in luminal (including HER2-positive luminal) compared to other breast cancers subtypes. Most studies show comparable efficacy in luminal A and B tumors, but there are hints of increased benefit in tumors with luminal B features [Goetz MP 2018].

Luminal B tumors are characterized by frequent DNA amplifications and chromosomal aberrations [Chin K 2006]. These involve oncogenes like ZNF703 [Sircoulomb F 2011, Holland DG 2011], which encodes a nuclear protein (whose expression is stimulated by oestrogen) that stimulates cell proliferation, inhibits ER-associated gene expression and induces E2F1-associated gene expression and leading to an increase in cancer stem cells, potentially contributing to endocrine-resistance. Another oncogene, SIX1, is frequently overexpressed in tumor initiating cells of luminal B tumors, and confers poor prognosis [Iwanaga R 2012].

The profiles of gene mutations differ sharply between luminal A and B tumors [Cancer Genome Atlas Network 2012]. Compared to luminal A tumors, luminal B breast cancers have higher frequency of TP53 mutations (29% versus 12%) and lower frequency of PIK3CA mutations (29% versus 45%), as well as low frequency of MAP3K1 and MAP2K4 mutations. GATA3 mutations, which have been shown to be associated with response to AIs [Ellis MJ 2012], occur with similar
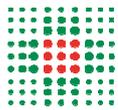
frequency in luminal A and B cancers (14 and 15% respectively), but differ in type, with a hotspot 2-base-pair deletion within intron 4 in the luminal A subtype and frame-shift mutations in exon 5 (DNA binding domain) in luminal B. Loss-of-function mutations of RUNX1, a transcription factor involved in ER genomic actions, or its dimerization partner CBFB, may induce endocrine resistance, and are associated with a luminal B phenotype [Ellis MJ 2012].

The retinoblastoma pathway is frequently disrupted in breast cancer, particularly in the basal-like and luminal B subtypes. Interpretation of study results is difficult because loss of heterozygosity of the RB1 tumor suppressor gene does not necessarily correlate with RB protein expression assessed by immunohistochemistry.

1.2 Investigational Agents

First-line treatment of metastatic luminal breast cancer

Endocrine therapy is the preferred first-line treatment for metastatic luminal breast cancer [Cardoso F 2018], and since the advent of CDK4/6 inhibitors a combination of an endocrine agent with a CDK4/6 inhibitor is commonly used as first-line (usually in combination with an AI, in patients naïve to AIs or relapsed >12 months after the end of adjuvant AIs, or with fulvestrant in patients relapsed while on or ≤12 months after the end of adjuvant AI) or second-line treatment (in combination with fulvestrant). According to the ESO-ESMO guidelines [Cardoso F 2018] the CDK4/6 inhibitors have a higher Magnitude of Clinical Benefit Scale (MCBS) score when used in the second line in combination with fulvestrant, where there is stronger evidence of health-related quality of life benefit, than when used in first-line. In some cases of advanced luminal breast cancer chemotherapy may be required, either at first metastatic relapse, or after failure of one line of endocrine therapy. This applies particularly to the luminal B breast cancer subtype, whose prognosis is worse, and responsiveness to endocrine therapy poorer, compared with the luminal A counterpart. In some clinical conditions also luminal A breast cancer might require chemotherapy. According to the Italian Association of Medical Oncology (AIOM) chemotherapy can be considered in the earlier lines of treatment in presence of signs of disease aggressiveness, such as short disease-free interval, elevated Ki67 (preferably, if available, on a metastatic biopsy), low

expression of hormone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms [AIOM breast cancer guidelines]. Nonetheless, it is unknown if treatment with CDK4/6 inhibitors plus endocrine therapy could replace chemotherapy in these circumstances in more aggressive luminal tumors, and some clinical trials are ongoing to compare the two treatments. Preclinical studies show a synergism between chemotherapy and some endocrine agents such as AIs and fulvestrant. Therefore, a combination of chemotherapy and endocrine therapy could be a further, potentially very active, treatment strategy in advanced luminal breast cancer.

The choice between a chemotherapy-based and a CDK4/6 inhibitor-based treatment remains particularly controversial in patients with doubtful endocrine sensitivity, e.g. due to a low expression of estrogen receptors (ERs), or of primary endocrine resistance, indicated by a short disease-free interval.

We plan to conduct a phase II group sequential response adaptive randomized clinical trial comparing a combination of chemotherapy plus endocrine therapy with a combination of CDK4/6 inhibitors plus endocrine therapy in patients with advanced HR-positive, HER2-negative breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option.

1.3 Preclinical Data

Chemo-endocrine therapy – preclinical data
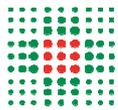
Endocrine agents and cytotoxic drugs have been often deemed potentially antagonistic, because cytotoxics act preferentially on proliferating cells, whereas endocrine agents act as cytostatics, thereby potentially reducing the efficacy of chemotherapy [Osborne CK 1981]. Preclinical studies on the combination of tamoxifen with cytotoxics yielded conflicting results, depending on the cytotoxic agent and the model system. 5-fluorouracil (5-FU) but not methotrexate proved synergistic with tamoxifen in breast cancer cell lines in one study [Benz C 1983], whereas in another study tamoxifen was shown to attenuate the cytotoxic potential of 5-fluorouracil (5-FU) and of doxorubicin, both on estrogen receptor positive and on estrogen receptor negative breast cancer cell lines [Hug V 1985]. Another study showed an antagonism between tamoxifen and some cytotoxics, such as melphalan and fluorouracil, and an additive effect with other cytotoxics, such as doxorubicin or 4-hydroxycyclophosphamide [Osborne CK 1989]. Antagonism was not due to tamoxifen cytostatic or anti-estrogen action, as it was present also in ER-negative breast cancer cells and in liver cells. A further study reported opposite results, with additive antitumor effect of concurrent tamoxifen and 5-FU, potentially explained by tamoxifen inhibition of thymidilate synthase, and less than additive effect of concurrent tamoxifen and doxorubicin, with increased expression of genes related to tamoxifen resistance by doxorubicin, in ER-positive cells [Kurebayashi J 2007]. Some contradictory results might be explained by an inhibitory effect of tamoxifen on the gp170 multidrug resistance glycoprotein, as tamoxifen was shown to interact synergistically with doxorubicin and vinblastin (and to increase vinblastine intracellular accumulation) only in cell lines that express gp170, while producing an antagonistic or at most additive interaction in cells without gp170 [Leonessa F 1994].

The effects of newer endocrine agents, which are devoid ER agonistic effects and hopefully of antagonism towards chemotherapy, have been less extensively studied.

Expression of ER is associated with reduced responsiveness to chemotherapy, as clearly shown by subgroup analysis of clinical trials of neoadjuvant chemotherapy. Preclinical studies have demonstrated that activation of ER by co-treatment with estradiol significantly reduced the therapeutic efficacy of paclitaxel in ER-positive breast cancer cell lines [Chang J 2012]. In the same experimental model, pretreatment with the selective estrogen receptor down-regulator fulvestrant sensitized ER-positive breast cancer cells to paclitaxel, reverting the ER-mediated
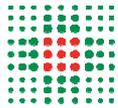
chemoresistance. The same effect was shown with other cytotoxic agents, such as vinorelbine and vinblastine [Sui M 2010]. In breast cancer cells expressing aromatase, the effect of a treatment with paclitaxel on cell growth was enhanced by co-treatment with the AI exemestane [Chen D 2004], independently of ER expression. On the other hand, treatment with docetaxel has been shown to decrease significantly intratumoral aromatase expression [Miyoshi Y 2004], providing a potential mechanism for synergism with AIs. Important additive or even synergistic effects with AIs have been reported for fluoropyrimidines. A combination of the AI fadrozole hydrochloride and 5-fluorouracil significantly increased the growth inhibition rate of the breast cancer cell line SK-BR-3, compared with treatment with each single agent [Tsunoda Y 2001]. Combinations of the oral fluoropyrimidines tegafur-uracil (UFT) or tegafur-gimeracil-oteracil (S-1) with the AI anastrozole additively inhibited the growth of MCF-7 breast cancer cell lines trasnfected with human aromatase gene [Nukatsuka M 2011]. In mice experiments, the combination of the two drugs significantly enhanced the antitumor efficacy, suppressing tumor growth for 2-4 times longer than monotherapy. Expression of ER in tumor tissue was markedly decreased after treatment with S-1 or S-1 plus anastrozole, but not after treatment with doxorubicin or paclitaxel, suggesting a further mechanism for a synergism between AIs and fluoropyrimidines [Nukatsuka M 2011]. Treatment with the short-acting selective estrogen receptor modulator arzoxifene between courses of chemotherapy in MCF-7 breast cancer xenografts has been shown to inhibit tumor repopulation, improving the treatment effectiveness [Wu L 2005].

CDK4/6-inhibitors – preclinical data

Three CDK4/6 inhibitors have been approved so far by the FDA and EMA for treatment of patients with breast cancer: palbociclib, ribociclib and abemaciclib. CDK4/6 inhibitors bind the ATP pockets of CDK 4 and 6 with high selectivity (abemaciclib has some selectivity also for CDK9) and inhibit Cyclin D – CDK4/6 dependent phosphorylation of the Rb protein. Rb is active when unphosphorylated and inhibits the E2F transcription factor that is responsible for the transcription of genes that execute the cell cycle. Phosphorylation of Rb leads to its inactivation, releasing its inhibitory effect on the cell cycle and favoring its progression from G1 into the S phase. CDK4/6

inhibitors therefore prevent inactivation of Rb and block cell cycle progression, producing Rb de-phosphorylation at specific serine residues as their pharmacodynamic hallmark [Sherr CJ 2016].

Among human breast cancer cell lines representative of the different breast cancer subtypes, the ER-positive, luminal ones are the most sensitive, along with some HER2-amplified cell lines with luminal features, whereas cell lines with basal features are the most resistant [Finn RS 2009]. Both endocrine-sensitive and endocrine-resistant lines may respond to CDK4/6 inhibitors [Petrossian K 2016]. High levels of cyclin D1 and of Rb, and low levels of p16 were predictors of sensitivity to palbociclib in vitro [Finn RS 2009] and in ex vivo studies on primary human tumor cultures [Dean JL 2012b].

Endocrine resistance often involves activation of the Cyclin D – CDK4/6 – Rb pathway and alterations of this pathway are common in luminal breast cancer. A combination of endocrine therapy and CDK4/6 inhibitors is therefore a relevant therapeutic option in these tumors. Preclinical studies show that the combination of palbociclib and tamoxifen is synergic in ER-positive breast cancer cell lines and palbociclib monotherapy is active in tamoxifen-resistant MCF7 cell lines [Finn RS 2009]. CDK4/6 inhibitors have also shown synergism with fulvestrant [Alves CL 2016, O'Brien 2014] and AI [Petrossian K 2016, O'Brien 2014].

1.4   Clinical Data to Date

Studies of chemo-endocrine therapy

Several studies comparing concomitant chemo-endocrine therapy with chemotherapy alone in first line metastatic breast cancer have been conducted in the past with the use of tamoxifen in combination with old chemotherapy regimens and have produced controversial results (reviewed in [Pritchard KI 2008]). Based on these findings and on studies of concomitant chemo-endocrine therapy with tamoxifen in the adjuvant setting [Pico C 2004, Albain KS 2009, Bedognetti D 2011, Del Mastro L Ann Oncol 2008; 19: 299-307, EBCTCG 2011], a sequential rather than concomitant strategy has become the standard of care.

Only limited experience exists with concomitant chemotherapy and newer endocrine agents in clinical trials. A phase II study assessed the activity of a combination of chemotherapy with FEC

(fluorouracil, doxorubicin and cyclophosphamide) for 6 cycles and concomitant exemestane 25 mg daily in postmenopausal patients with advanced breast cancer (ABC) [de la Haba-Rodriguez J 2010]. On 23 patients there were 10 partial and 7 complete clinical responses (overall response rate 74%), a clinical benefit (objective response or stable disease) in 87% of the patients, and a median time to progression of 13.7 months. There was one case of pulmonary embolism. Other phase II studies have evaluated the combination of chemotherapy and endocrine agents in the neoadjuvant setting, both in premenopausal women receiving a GnRH analog [Torrisi R 2007] as endocrine agent or a GnRH analog plus letrozole [Torrisi R 2008], and in postmenopausal women receiving an AI [Torrisi R 2008, Watanabe N 2010]. Overall, these studies yielded promising results, with objective clinical responses in about 75% of the patients and pathologic complete response rates ranging from 5 to 11%. The only randomized phase III trial reported in the literature compared a neoadjuvant chemotherapy with FEC (fluorouracil, doxorubicin and cyclophosphamide) for a median of 4 cycles with the same chemotherapy given concurrently with letrozole 2.5 mg daily [Mohammadianpanah M 2012]. On 101 patients randomized between the two arms, the pathologic complete response rate increased from 10% to 25% with the addition of letrozole, without worsening chemotherapy-related toxicity, although there were some added side effects typical of endocrine therapy, such as hot flushes.

The combination of fulvestrant and metronomic capecitabine (1500-2000 mg daily continuously) was evaluated in a phase II study in 41 post-menopausal women with advanced breast cancer, reporting a median PFS of 14.98 months (95%CI 7.26-NR), median TTP of 26.94 months (95%CI 7.26-NR) and clinical benefit rate of 58.5%, with excellent tolerability [Schwartzberg LS 2014].

Taken together, these evidences highlight the potential effectiveness of the concomitant administration of chemotherapy with the newer endocrine agents, such as AIs and fulvestrant, in patients with aggressive luminal Bbreast cancers.

**Studies of CDK4/6 inhibitors in combination with endocrine therapy**

All the CDK4/6 inhibitors that have undergone clinical development have been shown to improve results when combined with endocrine agents over endocrine therapy alone in patients with hormone receptor-positive, HER2-negative advanced breast cancer, both in first-line in

combination with an AI (in patients naïve to AI or relapsed >12 months after the end of adjuvant AIs) or with fulvestrant (in patients relapsed while on or ≤12 months after the end of adjuvant AI) and in second-line (in combination with fulvestrant).

In the first-line setting, the phase III trial PALOMA 2 compared placebo + letrozole with palbociclib + letrozole in 666 post-menopausal patients, not previously treated for advanced breast cancer, showing an increase in median PFS from 14.5 months in the placebo arm to 24.8 months in the palbociclib arm, with hazard ratio 0.58 (95% CI 0.46–0.72, p<0.000001) favoring palbociclib [Finn RS, 2016]. Similarly, the phase III study MONALEESA 2 compared placebo + letrozole with ribociclib + letrozole in 668 post-menopausal women with no prior systemic therapy for advanced disease, with a median duration of PFS not reached in the ribociclib group versus 14.7 months in the placebo group (hazard ratio 0.56; 95% CI, 0.43-0.72; p=0.000003) [Hortobagyi GN, 2016]. The phase III trial MONARCH 3 compared placebo + a non-steroidal AI (anastrozole or letrozole) with abemaciclib + a non-steroidal AI in 493 post-menopausal women with no prior systemic therapy for advanced disease, yielding a significant prolongation of PFS from a median of 14.7 months in the placebo arm to a median not reached in the abemaciclib arm (hazard ratio 0.54; 95% CI, 0.41-0.72; p=.000021) [Goetz MP, 2017].

In the second-line setting, the phase III study PALOMA-3 compared palbociclib plus fulvestrant versus placebo plus fulvestrant (with premenopausal women receiving also a GnRH agonist) in 521 women with HR-positive, HER2-negative advanced breast cancer progressing during or shortly after (≤12 months in the adjuvant and ≤1 month in the metastatic setting) prior endocrine therapy. Median PFS was 9.5 months with palbociclib versus 4.6 months with placebo, with hazard ratio 0.46 (95%CI 0.36-0.59, p<0.0001) [Turner NC, 2015]. The benefit in OS, a secondary endpoint, was not statistically significant (hazard ratio for death, 0.81; 95% CI, 0.64 to 1.03; P=0.09; absolute difference, 6.9 months) [Turner NC, 2018]. Similarly, the phase III study MONARCH 2 compared abemaciclib + fulvestrant versus placebo + fulvestrant (with premenopausal women receiving also a GnRH agonist) in 669 women who had progressed while receiving neo/adjuvant endocrine therapy, ≤12 months after the end of adjuvant endocrine therapy, or while receiving first-line endocrine therapy for advanced disease, showing a median PFS of 16.4 months with abemaciclib

versus 9.3 months with placebo (hazard ratio 0.553; 95%CI 0.449-0.681; p=.001) [Sledge GW Jr, 2017].

The phase III trial MONALEESA-3 compared ribociclib + fulvestrant versus placebo + fulvestrant in 484 post-menopausal women with HR-positive, HER2-negative advanced breast cancer who were treatment naïve or had received up to one line of prior endocrine therapy in the advanced setting. Median PFS was 20.5 months with ribociclib versus 12.8 months with placebo (hazard ratio 0.593; 95% CI 0.480-0.732; p=.001) [Slamon DJ, 2018]. Similar results were observed in the subgroups of patients who were treatment naïve and in those pretreated.

The phase III MONALEESA-7 trial compared ribociclib + endocrine therapy (either tamoxifen or a non-steroidal aromatase inhibitor, all with goserelin) with placebo + endocrine therapy in 672 pre-menopausal women not previously treated with endocrine therapy for advanced disease. The median PFS was 23·8 months in the ribociclib group compared with 13·0 months in the placebo group (hazard ratio 0.55, 95%CI 0.44–0.69; p<0·0001) [Tripathy D, 2018].

All these trials show a clinically relevant improvement in PFS with the addition of a CDK4/6 inhibitor to endocrine therapy, which is generally accompanied by improvements in other endpoints such as the objective response rate. This highlights the potential of these regimens to substitute chemotherapy in cases of aggressive luminal tumors. While clinical trials comparing these two treatment modalities are ongoing, an indirect comparisons between CDK4/6 inhibitors and chemotherapeutic drugs done by means of a network meta-analysis shows improvements in PFS with CDK4/6 inhibitors compared to several chemotherapeutic agents [Wilson FR, 2017].

1.5   Rationale and Risk/Benefits

The best treatment approach for patients with aggressive luminal breast canceris still controversial and studies comparing endocrine therapy plus CDK4/6 inhibitors with chemotherapy are ongoing. Preclinical evidences of synergism between chemotherapy and newer endocrine agents suggest that a combination of chemotherapy plus endocrine therapy could be a further effective treatment for theses patients. We therefore plan a randomized clinical trial to test the superiority of a combination of chemotherapy plus endocrine therapy (experimental arm) versus the

combination of CDK4/6 inhibitors plus endocrine therapy (standard arm), in patients with advanced luminal breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option. Patients in the experimental arm will receive chemotherapy, with a regimen at the discretion of the treating physician, in combination with an endocrine agent (either AI or fulvestrant) started (at the discretion of the treating physician) concomitantly with chemotherapy or sequentially after 4-6 months of chemotherapy and continued until disease progression or toxicity or patient refusal.

In case of superiority of the combination of chemotherapy plus endocrine therapy, this should be considered the first choise treatment for aggressive luminal tumors. If superiority is not established, the less toxic treatment should be considered as first choise. The comparison between concomitant versus sequential administration of chemotherapy and endocrine therapy will be a secondary exploratory endpoint.

The trial allows patients entered in the chemotherapy plus endocrine therapy arm to receive CDK4/6 inhibitors in subsequent lines, recommending cross over, therefore does not deny to these patients the opportunity to receive CDK4/6 inihibitors.

Given the low toxicity profile of the new endocrine agents, no relevant increase in toxicity is expected in the chemotherapy arm, but thromboembolic events, potentially favored both by chemotherapy and endocrine therapy, will be specifically monitored.

2. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

The study will test the hypothesis of superiority of a combination of chemotherapy plus endocrine therapy (AI or fulvestrant) compared with cyclin-dependent kinase 4/6 inhibitor plus endocrine therapy (AI or fulvestrant) in patients with locally advanced and metastatic HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.

2.1 Primary Objective

To compare the efficacy of a combination of chemotherapy plus endocrine therapy versus CDK4/6 inhibitors plus endocrine therapy in terms of PFS.

The statistical hypothesis is that of superiority of the combination of chemotherapy plus endocrine therapy compared with CDK4/6 inhibitor plus endocrine therapy in terms of PFS (see the section 11. Statistical Considerations).

2.2 Secondary Objective

To compare between the two treatment arms:

- quality of life (EORTC QLQ-C30 and QLQ-BR23)
- toxicity (CTCAE version 5.0)
- time to treatment failure (TTF)
- best response rate
- duration of response
- clinical benefit rate
- overall survival
- PFS and clinical benefit with the subsequent line of treatment after cross-over: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy
- To assess correlative biomarkers of response to chemotherapy and CDK4/6 inhibitors:

- o tissue markers (on the primary tumor and / or metastatic tissue)

- o circulating markers (e.g. CTCs, ctDNA)

To compare, within the experimental arm, PFS with concomitant versus sequential administration of chemotherapy and endocrine therapy (exploratory endpoint)

2.3 Endpoints /Outcome measures

See sections 2.4 and 2.5 for outcome measures for primary and secondary objectives.

Both the evaluation of clinical outcomes and quality of life, and the collection of blood samples, will take place at baseline and every 3 months during therapy, until evidence of disease progression.

2.4 Primary endpoint/outcome

- PFS: time from randomization until first disease progression or death; disease progression is defined according to Response Evaluation Criteria In Solid Tumours (RECIST) version 1.1 (see Appendix D)

2.5 Secondary endpoints/outcomes

- quality of life:
    - o EORTC QLQ-C30 Version 3.0
    - o QLQ-BR23 (breast cancer specific)
- toxicity: Common Termonology Criteria for Adverse Events (CTCAE) version 5.0 (see Appendix B)
- TTF: the time from randomization to treatment discontinuation for any reason, including disease progression, treatment toxicity, patient refusal or death.
- best objective (partial or complete) response rate according to RECIST 1.1 (see Appendix D)
- duration of response: time from documentation of tumor response to disease progression
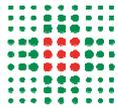
- clinical benefit rate (CBR): the percentage of patients who achieved complete response, partial response or stable disease lasting longer than 24 weeks

- overall survival (OS): time from randomization until death for any cause

- PFS and clinical benefit with the subsequent line of treatment after cross-over: as above, calculated from the date of start of the subsequent treatment line

- correlative biomarkers: assessed on baseline tumor specimens (from primary tumor or metastatic biopsies) and blood samples collected at baseline and at different timepoints until evidence of disease progression.

3.   STUDY DESIGN

3.1   Summary of Trial Design

This is a prospective, open label, multicenter, phase 2, group sequential response adaptive randomized trial, comparing two combination treatments for locally advanced or metastatic HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness:

  - Arm A: concomitant CDK4/6 inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant).

  - Arm B: chemotherapy plus endocrine therapy (AI or fulvestrant). The chemotherapy regimen will be at the discretion of the treating physician and will be administered for at least 4-6 months (unless there is toxicity or disease progression). The endocrine agent can be started concomitantly with chemotherapy or sequentially, after stopping chemotherapy.

Treatments will continue until disease progression or toxicity or patient refusal.

Cross-over to the other treatment arm is suggested (but not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy within the study, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy within the study.

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Istituto di Ricovero e Cura a Carattere Scientifico

Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0

Patients will be enrolled and randomized after the screening period (which should last no longer than 28 days), will be followed during treatment with visits for evaluation of toxicity at each cycle and with imaging for disease evaluation at least every 3 months until disease progression or until exit from the study for toxicity or patient refusal.

Patients' enrolment is expected to occur over a period of 23 months, with further 16 months of follow up.

Trial scheme

Disease: **Treatment**

Luminal (HER2-negative) locally advanced or **metastatic breast cancer (ABC)**, with features of aggressiveness

Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy

Permuted Block Randomization (until two relapses are experienced in both arms) →

Response Adaptive Randomization (the allocation probabilities updating process will take place after 105 and 128 patients are enrolled along with interim analysis on efficacy allowing for early stopping of the trial)

Arm B: concomitant chemotherapy plus endocrine therapy

Treatments:
CT:
- anthracyclines + taxanes
- taxanes
- anthracyclines
- fluoropyrimidines
- others

ET:
- non-steroidal AI
- steroidal AI
- fulvestrant

CKK4/6 inhibitor:
- palbociclib
- ribociclib
- abemaciclib

Treatments will continue until disease progression or toxicity or patient refusal

Cross-over is encouraged (although not mandatory)

3.2   End of trial definition

The end of trial will be the date of the last visit of the last subject, or 1 month after the last data capture, whichever occurs first. The Coordinating Centre (CC) or Promoter will notify the IEC(s) that the trial has ended and a summary of the clinical trial report will be provided within 12 months of the end of trial.

4.   STUDY POPULATION

The study will enroll postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not), with HR-positive, HER2-negative, breast cancer, with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.

Patients must have baseline evaluations performed prior to the first dose of study drug and must fulfill all eligibility criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.1   Inclusion Criteria

1. Histological diagnosis of HR-positive (ER ≥10% of tumor cells), HER2-negative (according to ASCO guidelines 2018) breast cancer, determined by local laboratory on most recent available tumor tissue.

2. Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as advanced breast cancer, ABC).

3. At least one of the following signs of disease aggressiveness:

    a. the main criteria are a low expression of ER (10% ≤ ER < 50%) and/or or a relapse while on the first 2 years of adjuvant endocrine therapy or disease progression within the first 6 months of first-line endocrine therapy for ABC.

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Istituto di Ricovero e Cura a Carattere Scientifico

Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0

b. Other tumor characteristics of aggressiveness that make the patient potentially candidate to chemotherapy, according to the guidelines of the Italian Association of Medical Oncology [AIOM guidelines 2017], such as: elevated Ki67 (preferably documented, if available, on a metastatic biopsy), low expression of hormone receptors (e.g. progesterone receptor <20%), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms; these patients are eligible if chemotherapy is considered a suitable option by the treating physician.

4. Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not). Postmenopausal status is defined as:

    a. bilateral, surgical oophorectomy

    b. age ≥60 years

    c. age <60 years, with amenorrhea >12 months and follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol concentrations within postmenopausal range

    d. age <60 years and previous simple hysterectomy, with FSH, LH and estradiol levels within the post-menopausal range at two consecutive assessments two weeks apart.

5. Measurable disease according to RECIST 1.1 criteria or non-measurable but evaluable lesions.

6. Any prior adjuvant chemotherapy or endocrine therapy

7. No prior chemotherapy for ABC.

8. Up to one prior line of endocrine therapy for ABC.

9. Age ≥ 18 years.

10. Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).

11. Adequate organ (renal, hepatic, bone marrow, cardiac) functions.

12. Female participants of child-bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.

13. Participant is willing and able to give informed consent for participation in the study

4.2   Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

1. Any prior chemotherapy or CDK4/6 inhibitor for ABC.

2. More than 1 prior line of endocrine therapy for ABC.

3. Patients who have not recovered from adverse events (AEs) due to prior therapies to grade ≤1 (excluding alopecia).

4. Active central nervous system metastases.

5. History of allergic reactions attributed to compounds of similar chemical or biologic composition to the drugs used in the study.

6. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

7. Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.

5.   STUDY PROCEDURES

All on-study visit procedures are allowed a window of ±8 days unless otherwise noted. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.
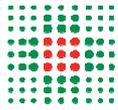
See for schedule of procedures Appendix C.

5.1   Informed Consent

A written, signed, informed consent form (ICF) must be obtained before any study-specific assessments are initiated.

Written and verbal versions of the participant information and Informed consent will be presented to the participants detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site.

5.2   Screening and Eligibility Assessments

The Screening procedures and assessments must be completed within 28 days of Randomization.

Data to be collected during the screening period include:

- Demographics: the date of birth, gender, race.
- Complete medical history: details of any history of prior or concurrent diseases or surgical interventions.
- Confirmation and documentation of HER2 and ER/PgR status on the most recent tumor tissue

- Baseline clinical conditions, including symptoms assessment.

- History of prior treatments and any residual toxicity relating to prior treatment if applicable.

- Concomitant Medication: baseline medications taken within 28 days of Day 1, starting date and prescribing indication.

- Physical Examination, with description (and measurement of the main diameter when feasible) of superficial tumor lesions (or picture if deemed useful).

- ECOG-PS, vital signs (including at least body temperature, resting blood pressure and cardiac frequency), height and weight.

- 12-lead electrocardiogram (ECG), cardiological examination and/or cardiac function assessment by ECHO or MUGA if clinically indicated (recommended for cardiotoxic drugs).

- Laboratory Tests:
    - complete blood count (CBC) with differential and platelet count
    - blood chemistry assessment including renal and liver function tests (e.g. serum creatinine, potassium, sodium, chloride, calcium, ALAT/SGPT, total bilirubin, alkaline phosphatase, total proteins and albumin)
    - urinalysis if clinically indicated.
    - serum CA15/3.
    - coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR), if clinically indicated.

- Serum or urine pregnancy test within one week prior to the start of study drug for women of child bearing potential.

- Tumor evaluation: CT scan of the chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted. All other clinically indicated examinations (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis).

- Bone scan if clinically indicated (recommended if bone pain is present or bone lesions are found at CT)

- Availability of formalin-fixed, paraffin-embedded tumor specimen (from primary tumor or, when available, from a metastatic biopsy)

- Blood sample for Circulating Tumor Cells (CTCs) analysis

- Blood samples for circulating markers (other than CTCs)

- Quality of life Questionaires: EORTC QLQ-C30 Version 3.0, QLQ-BR23 (breast cancer specific)

5.3   Baseline Assessments (screening day -28 to 0)

All screening and eligibility assessments (see paragraph 5.2) performed within 28 days from randomization are considered as baseline assessments.

5.4   Randomization

Patients will be randomized on a 1:1 allocation rate according to block randomization (block of size two) until two events (i.e. disease progression or death) are experienced in both arms and then according to a group sequential DBCD [Hu F 2004] targeting Neyman allocation for time to event data [Zhang L 2007]. The allocation probabilities will be computed at the end of the block randomization and updated after 70% and 85% of the maximum 150 patients are enrolled (i.e. 105 and 128 patients). Central Sseparated randomization lists will be generated at the start of the trial and the at each halt. The chemotherapy, CDK4/6 inhibitor and endocrine agents will be decidedchosen by the treating physician for each patient before randomization.

Treatment administration should begin within 72 hours of the date of randomization.

No blinding is planned.

5.5   Randomization procedure

All patients for whom eligibility criteria have been verified will be randomized by the Biostatistics and Clinical Trial Unit of IRST (Coordinating Centre, CC).

All patients must be randomized following the standard IRST procedure prior to initiation of study therapy sending by fax the registration form to:
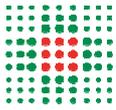
Unità di Biostatistica e Sperimentazioni Cliniche
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST)
c/o Ospedale Civile Santa Maria delle Croci
Dipartimento Oncologia e Ematologia
viale Randi n°5 - 48121 Ravenna (RA)
Tel: 0544 285813, Fax: 0544 285330,
cc.ubsc@irst.emr.it

All working days from Monday to Friday, from 9 AM to 16 PM.

Further detailed information will be sent to participating centres and will also be included in the Investigator Site File.

5.6   Assessments during treatment period (cycle duration 21 or 28 days)

<u>Before each chemotherapy or CDK4/6 inhibitor cycle</u> (within 36 hours prior to treatment administration) the following evaluations must be performed:

- Interim medical history, including tumor-related signs and symptoms and new medical conditions

- Assessment of AEs (CTCAE version 5.0, Appendix B);

- Assessment of compliance with study drugs (oral chemotherapy, CDK4/6 inhibitor and/or endocrine therapy)

- Assessment of concomitant medications (in particular if the patient started new medications, registering dosage, route of administration, start and end date and the clinical indication)

- Physical examination.

- ECOG-PS, vital signs (including at least resting blood pressure and cardiac frequency), weight.

- Laboratory Tests: CBC and serum chemistry

<u>Every 3 months</u> (every 4 cycles for 21-day chemotherapy regimens, every 3 cycles for 28-day chemotherapy or CDK4/6 inhibitor regimens) the following evaluations must be performed:

- Physical Examination, with description (and measurement of the main diameter when feasible) of superficial tumor lesions
- Tumor assessment with the same radiological exams used to document the disease at baseline:
    - CT scan of the chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted.
    - Other exams for tumor assessment if positive at baseline (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis) or as clinically indicated (suspected disease progression at new sites)
- Serum CA15/3
- Quality of life questionnaires:
    - EORTC QLQ-C30 Version 3.0
    - QLQ-BR23 (breast cancer specific)
- Blood samples for circulating markers (other than CTCs)
- Blood samples for CTCs are to be collected 6-8 weeks after the beginning of study treatment, and at the end of treatment.

5.7 End of treatment assessments (within 30 days of last treatment administration)

The following assessments will be completed within 30 days of the patient's last treatment administration:

- Interim medical history, including tumor-related signs and symptoms and new medical conditions
- Assessment of AEs (CTCAE version 5.0, Appendix B);
- Assessment of compliance with study drugs (oral chemotherapy, CDK4/6 inhibitors and/or endocrine therapy)
- Assessment of concomitant medications (in particular if the patient started new

medications, registering dosage, route of administration, start and end date and the clinical indication)

- Physical examination, with description (and measurement of the main diameter when feasible) or picture of superficial tumor lesions.

- ECOG-PS, vital signs (including at least resting blood pressure and cardiac frequency), weight.

- 12-lead electrocardiogram (ECG), cardiological examination and/or cardiac function assessment by ECHO or MUGA if clinically indicated (recommended for cardiotoxic drugs).

- Laboratory Tests: CBC and serum chemistry; urinalysis if clinically indicated.

- Tumor evaluation is required for patients who discontinue study treatment before the first scheduled post-baseline tumor assessment and for patients whose previous tumor assessment did not demonstrate PD and was done more than 2 months prior to the end of treatment visit, with the same radiological exams used to document the disease at baseline:

  o CT scan of chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted.

  o Other exams for tumor assessment if positive at baseline (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis) or as clinically indicated (suspected disease progression at new sites)

- Serum CA15/3

- Quality of life questionnaires for patients whose previous assessment was done more than 2 months prior to end of treatment visit:

  o EORTC QLQ-C30 Version 3.0

  o QLQ-BR23 (breast cancer specific)

- Blood samples for circulating markers (other than CTCs).

5.8   Follow-up visits

After the EOT visit, all the patients will be followed according to clinical practice. Data on response and TTP to the immediate subsequent line of treatment (particularly in case of cross-over) must be registered, along with survival.

For survival **data** phone contacts are acceptable.

5.9   End of study

The end of study is the date of withdrawal of consent, death of the patient or the date of closure

of the study, whichever comes first.

5.10   Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if necessary for any reason including:

- Failure of the treatment strategy because of disease progression
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Significant non-compliance with treatment regimen or study requirements
- An AE which requires discontinuation of all study medications or results in inability to continue to comply with study procedures
- Pregnancy
- Lost to follow up

Consent withdrawal will not result in exclusion of the data already collected for that participant from analysis. The reason for withdrawal will be recorded in the eCRF.
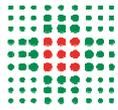
5.11 Source Data

Source documents are original documents, data, and records from which participants' eCRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the eCRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

eCRF entries will be considered source data if the eCRF is the site of the original recording (e.g., there is no other written or electronic record of data).

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

Direct access will be granted to authorised representatives from the promotor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

6. STUDY TREATMENT

All the investigational medicinal products used in the clinical trial are authorized for use in ABC, although the concomitant administration of endocrine agents and chemotherapy is not standard practice.

Any cytotoxic or endocrine agent to which a patient was exposed in the adjuvant setting may be administered within this study only if PD occurred at least 12 months after the end of that drug.

Chemotherapy regimens are at the discretion of the treating physician, based also on patient's features and preferences.

Possible chemotherapy regimen**s** are:

- based on anthracycline and taxane
- taxane-based (without anthracycline)
- anthracycline-based (without taxane)
- based on capecitabine or other fluoropyrimidines
- other

Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard" per each individual agent's prescribing information. Dosage adjustments during treatment are at the discretion of the treating physician, according to clinical practice.

<u>Endocrine therapy:</u>

- non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI

- non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed while on adjuvant steroidal AI

- steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed on adjuvant non-steroidal AI

- fulvestrant in women not pretreated with fulvestrant for advanced disease

<u>CDK4/6 inhibitor:</u>

- palbociclib

- ribociclib

- abemaciclib

6.1 Description of Study Treatment

Possible endocrine treatments are:

- non-steroidal AIs:
  - anastrozole 1 mg daily continuously
  - letrozole 2.5 mg daily continuously

- steroidal AI:
  - exemestane 25 mg daily continuously

- fulvestrant, 500 mg i.m. on days 1, 15, 29 and then every 4 weeks

<u>Exemestane</u> is metabolised through P450-cytochrome enzymes, mainly CYP3A4 but also others (CYP1A1/2, CYP4A11, CYP3A5, CYP2B6, CYP2A6, CYP2C8, CYP2C9 and CYP2C19), as well as through aldoketoreductases, and does not inhibit any of the major CYP isoenzymes. In a clinical
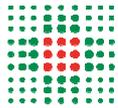
pharmacokinetic study, the specific inhibition of CYP3A4 by ketoconazole showed no significant effects on the pharmacokinetics of exemestane. In an interaction study with rifampicin, a potent CYP450 inducer, the AUC of exemestane was reduced by 54% and Cmax by 41%. The co-administration of drugs, such as rifampicin, anticonvulsants (e.g., phenytoin and carbamazepine) and herbal preparations containing hypericum perforatum (St John's Wort) known to induce CYP3A4 may therefore reduce the efficacy of exemestane.

Exemestane should be used cautiously with drugs that are metabolised via CYP3A4 and have a narrow therapeutic window.

Metabolism of <u>letrozole</u> is partly mediated via CYP2A6 and CYP3A4. Cimetidine, a weak, unspecific inhibitor of CYP450 enzymes, did not affect the plasma concentrations of letrozole. The effect of potent CYP450 inhibitors is unknown. In vitro, letrozole inhibits the cytochrome P450 isoenzymes 2A6 and, moderately, 2C19, but the clinical relevance is unknown. Caution is therefore indicated when giving letrozole concomitantly with medicinal products whose elimination is mainly dependent on these isoenzymes and whose therapeutic index is narrow (e.g. phenytoin, clopidrogel).

<u>Anastrozole</u> inhibits CYPs 1A2, 2C8/9 and 3A4 in vitro. Clinical studies with antipyrine and warfarin showed that anastrozole at a 1 mg dose did not significantly inhibit the metabolism of antipyrine and R– and S-warfarin indicating the co-administration of anastrozole with other medicinal products is unlikely to result in clinically significant medicinal product interactions mediated by CYP enzymes. The enzymes mediating metabolism of anastrozole have not been identified. Cimetidine, a weak, unspecific inhibitor of CYP enzymes, did not affect the plasma concentrations of anastrozole. The effect of potent CYP inhibitors is unknown. A review of the clinical trial safety database did not reveal evidence of clinically significant interaction in patients treated with anastrozole who also received other commonly prescribed medicinal products.

<u>Fulvestrant</u> metabolism involves multiple transformations, similarly to endogenous steroids, and cytochrome p450 3A4 (CYP3A4) appears involved in its oxidation. Despite that, there are no know

interactions with other drugs, including inducers and inhibitors of CYP3A4, and fulvestrant does not inhibit other CYP enzymes.

The CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib are primarily metabolized by CYP3A and sulfotransferase (SULT) enzyme SULT2A1, and are time-dependent inhibitors of CYP3A.

Avoid the concomitant use of CDK4/6 inhibitors with strong CYP3A inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, posaconazole, voriconazole, boceprevir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir, conivaptan, telithromycin, nefazodone; grapefruit or grapefruit juice), which lead to increased plasma exposure of CDK4/6 inhibitors. If coadministration of a strong CYP3A inhibitor cannot be avoided, reduce the dose of CDK4/6 inhibitors.

Avoid concomitant use of CDK4/6 inhibitors with strong CYP3A inducers (e.g., phenytoin, rifampin, carbamazepine, enzalutamide, and St John's Wort), which decrease the plasma exposure of CDK4/6 inhibitors.

The dose of drugs which are CYP3A substrates with a narrow therapeutic index (e.g. midazolam, alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus) may need to be reduced, as CDK4/6 inhibitors may increase their exposure.

The use of ribociclib must be avoided in concomitance with drugs with a known potential to prolong QT such as antiarrhythmic medicines (including, but not limited to amiodarone, disopyramide, procainamide, quinidine and sotalol), and other drugs that are known to prolong the QT interval (including, but not limited to, chloroquine, halofantrine, clarithromycin, haloperidol, methadone, moxifloxacin, bepridil, pimozide and ondansetron).

Allowed chemotherapy regimens are all those registered for metastatic breast cancer including: anthracycline plus taxane, taxane-based (without anthracycline), anthracycline-based (without taxane), capecitabine- (or other fluoropyrimidines)-based, and others.

See the prescribing information of each drug for further informations.

6.2   Supply of study treatment

Commercial batches for all drugs will be used for the purposes of this study and will be relabeled. Labels will be prepared in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines. The labels will fulfill GMP Annex 13 requirements for labelling. Label text will be in local language.

Each drug package will have an investigational product label permanently affixed to the outside stating that the material is for clinical trial/investigational use only and should be kept out of reach of children. The label will include the dosing instructions and a space for the enrolment code (E-code) to be completed at the time of dispensing.

For oral drugs the label will include also the following information:

- blank lines for quantity of tablets to be taken

- date of dispensing

- Instructions stating that the tablets should be taken at approximately the same time each morning and/ or evening

All study drugs are included in the Italian national formulary and reimbursed by the National Health System.

Product description: see the prescribing information leaflet for each drug used

Solution preparation see the prescribing information leaflet for each drug used

Storage requirements: see the prescribing information leaflet for each drug used

Stability: see the prescribing information leaflet for each drug used

Route of administration: see the prescribing information leaflet for each drug used, and the literature articles describing the chemotherapy regimen used

Expected AEs: see the prescribing information leaflet for each drug used, and the literature articles describing the chemotherapy regimen used.

Please see the Investigator's Brochure for more details.
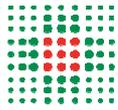
6.3 Compliance with Study Treatment

Regarding the oral study treatments, the participants will be instructed to return all unused or part-used medication and packaging from used oral medication at each visit. The patient compliance will be assessed by the investigator and/or other study personnel at each patient visit, using pill counts and information provided by the patient and/or caregiver.

The Investigator can decide to withdraw the patient from the study treatment if his/her compliance is unsatisfactory.

6.4 Accountability of the Study Treatment

Commercial batches for all drugs will be used for the purposes of this study, according to the indications registered by AIFA.

All treatments will be acquired by each participating center pharmacy and reimbursed by SSN.

All participating centers will provide separate drug and patient accountability of all study medication. All movements of study medication will be documented. The patient will be asked to bring all unused medication and used/packaging back to the clinic at each visit wher it will be returned to the pharmacy.

6.5 Concomitant Medication

Standard premedications for the administration of the cytotoxic chemotherapy will be employed in the study. For chemotherapy, recommendations made in the locally approved label should be followed for each agent.

Bisphonates or a RANK ligand inhibitor may be given according to their product license and routine clinical practice at the investigator's discretion.

Particular attention must be given to concomitant medications in patients receiving CDK4/6 inhibitors, which have e strong potential for drug-drug interactions.

Any medication, other than the study medication taken during the study will be recorded in the eCRF.
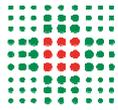
7. DOSING DELAYS/DOSE MODIFICATIONS

7.1 Study treatment modifications

Any toxicity observed during the study can be managed by dose delay, reduction and/or interruption if deemed appropriate by the Investigator. Suggested dose modifications are those recommended by the prescribing information leaflets of each drug (in particular for CDK4/6 inhibitors and chemotherapeutic agents) and/or standard dose modifications rules for chemotherapy regimens.

7.2 Monitoring and Toxicity Management

Each patient receiving at least one administration of study treatments will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and reports of AEs reported to the investigator by patients.

Each patient will be assessed periodically for the development of any toxicity as outlined in Section 5. Study Procedures. Toxicity will be assessed according to the NCI CTCAE v5.0.

8. CORRELATIVE STUDIES

With biological correlative studies, we will assess predictors of response to chemotherapy and CDK4/6 inhibitors in archival specimens from the primary tumor or from metastatic samples, as well as in blood samples collected at baseline and then repeated at different time points during therapy.

As this study allows the use of different chemotherapeutic agents and regimens, at the discretion of the treating physician, biological assessments will focus mainly on pathways involved in the response to chemotherapy in general, as opposed to features portending responsiveness to specific agents. Three main pathways are identified as important to this aim: p53, Rb, and p38/JNK MAPK pathways. The Cyclin D – CDK4/6 – Rb pathway is also crucial for responsiveness to CDK4/6 inhibitors.
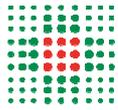
8.1   Background and aim of the study

p53 pathway

The p53 pathway is designed to respond to a wide variety of intrinsic and extrinsic cellular stress signals [Levine AJ, 2006], monitoring the maintenance of homeostasis and the fidelity of cellular duplication processes to prevent the development of abnormal cellular clones, and acting by the induction of a set of responses ranging from cell cycle arrest to allow damage repair, to apoptosis or cellular senescence when the damage is too large to be repaired.

The pathway is activated by input signals, including DNA damage or damage to the mitotic spindle from cytotoxic drugs. These act through upstream mediators such as the ATM (Ataxia Teleangectasia Mutated) and the ATR (Ataxia Telangiectasia and Rad3-related) serine/threonine protein kinases, activating the core circuit, which is regulated by an auto-regulatory loop involving p53 and MDM2. p53 then acts as a transcription factor, binding to p53-responsive elements in the genome, and leading to three primary responses: cell cycle arrest, cellular senescence and apoptosis, and other secondary responses [Levine AJ, 2006]. p53 can than exert opposing effects on biological processes (antagonistic bifunctionality), including pro-survival and pro-apoptotic activities [Aylon Y, 2016].

Mutations in the TP53 gene (encoding the p53 tumor suppressor protein) are found in more than 50% of cancers, and an even larger proportion of cancers harbors mutations in at least one of the genes of the p53 pathway [Stracquadanio G, 2016]. According to data from the Catalogue Of Somatic Mutations In Cancer (COSMIC), of the 67 autosomal genes attributed to the p53 pathway, 15 (23.4%) have been shown to harbor somatic, causal mutations in at least one type of cancer, including TP53, ATM, ATR, MDM2, MDM4, CDKN2A (p16INK4a/p14ARF), CDKN1A (p21CIP1), CCND1/2/3 (Cyclin D1/2/3), CDK4, CDK6, CCNE1/2 (Cyclin E1/2), FAS, CASP8, PTEN, TSC2.

TP53 mutations lead first of all to loss of the oncosuppressive properties (loss of function, LOF), but may sometimes lead to gain of novel oncogenic functions (gain of function, GOF) [Muller PA, 2014; Muller PA, 2013; Walerych D, 2015].
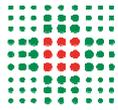
The association between mutant p53 and chemoresistance is unclear in epithelial tumors [Brown JM, 2005]. Contradictory results have emerged from studies in breast cancer, where some reports found no association [Bonnefoi H, 2011; Mathieu MC, 1995; Makris A, 1995], some showed better responses with wild-type p53 [Aas T, 1996; Berns EM, 2000; Kröger N, 2006; Rahko E, 2003; Chrisanthar R, 2011], and other showed better responses with mutant p53 [Bertheau P, 2002; Bertheau P, 2007].

More consistent are results indicating mutant p53 as predictor of endocrine-resistance, either to tamoxifen [Berns EM, 2000; Kim HS, 2010] and to aromatase inhibitors [Yamamoto M, 2014; Garimella V, 2014; Jia XQ, 2015].

Discrepancies on the effect of mutant p53 on treatment response may be at least in part related to the different methods of assessment of p53 status. While next generation sequencing is certainly the most accurate method, p53 overexpression detected by immunohistochemistry has often been considered equivalent to the presence of a TP53 mutation, although truncating mutations do not lead to stabilization/overexpression of p53, and stabilization of p53 is not always due to a mutation but may be due to a transient p53 activations [Bertheau P, 2013].

Recent preclinical works [Jackson JG, 2012; Varna M, 2009] highlight the induction of senescence as the main response of p53 wild-type breast cancer to drugs such as doxorubicin. On the contrary, p53 mutant (or p53 null) breast cancer cells exposed to doxorubicin proceed through the cell cycle, and the DNA damage induced by the drug causes aberrant mitoses which ultimately lead to apoptosis.

Responses of p53 wild-type cancer cells to doxorubicin may differ according to drug dose and the level of DNA damage [Udden SM, 2014]: lower doses have been shown to activate p53, leading to induction of p21 and consequent G2 arrest and senescence, while higher doses induced apoptosis.

Rb pathway

The retinoblastoma tumor suppressor gene product (Rb) is a major controller of cell cycle progression and has been called the "guardian of the cell cycle restriction point". Major players of cell cycle are the serin/threonin kinases CDKs [Malumbres M, 2014], their activating regulatory subunits cyclins [Ma Z et al. 2013], the so-called pocket proteins retinoblastoma (Rb, product of
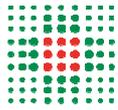
the retinoblastoma tumor suppressor gene RB1) and retinoblastoma-like proteins p107 (RBL1) and p130 (RBL2) [Dick FA et al, 2013], and the E2F family of transcription factors [Chen HZ et al. 2009].

Two main classes of CDK inhibitors (cyclin kinase inhibitors, CKI) exist: the inhibitor of CDK4 (INK4) family, including p16INK4A, p15INK4B, p18INK4C, and p19INK4D, specifically blocking the formation of cyclin D-CDK4/6 complexes [Sherr CJ and Roberts JM, 1999], and the CDK-interacting protein/kinase inhibitory protein (CIP/KIP) family, including p21Cip1, p27Kip1, and p57Kip2, acting on all cyclin-CDK complexes, with prevalent inhibitory but sometimes activatory effects, in particular activating cyclin D-CDK4/6 complexes [LaBaer J et al. 1997].

Genomic aberrations or altered expression of molecules of the cyclins-CDKs-Rb pathway are frequent in breast cancer. According to The Cancer Genome Atlas (TCGA) [Cancer Genome Atlas Network 2012], the gene encoding for cyclin D1 (CCND1) is amplified in 29% and 58% of luminal A and B tumors, respectively, and in 38% of HER2-enriched tumors, whereas that encoding cyclin E (CCNE1) is amplified in 9% of triple negative tumors. Cyclin D1 overexpression, detected by immunohistochemistry in up to half of breast cancers, is even more frequent than CCND1 amplification, implying that other mechanisms can lead to deregulated expression [Bartkova J et al. 1994; Gillett C et al. 1994].

Amplification of CDK4 gene is reported in about 15% of breast cancers, results in protein overexpression, and is associated with high Ki-67 labeling index [An HX et al. 1999]. It occurs in 14% of luminal A, 25% of luminal B, and 24% of HER2-enriched cancers [Cancer Genome Atlas Network 2012].

Loss of Rb function in stem or progenitor cells is often a key event in neoplastic transformation [Sage J, 2015] and is accompanied by epithelial-mesenchymal transition [Arima Y et al. 2012]. According to TCGA data, loss of Rb due to RB1 gene deletion occurs overall in 2-4% of breast cancers, and loss due to RB1 truncating mutations in 1-3% [Johnson J et al. 2016], depending on breast cancer subtype [Cancer Genome Atlas Network 2012]. This leads to constitutive activation of E2F and induction of cyclin E and CDK2, independently from cyclin D-CDK4/6 activation, and is frequently accompanied by upregulation of p16 [Subhawong AP et al. 2009] due to a feedback loop [Kotake Y et al. 2007]. Other authors report higher frequencies of Rb loss of heterozygosity, correlating with low mRNA expression, particularly in triple-negative but also in luminal B breast
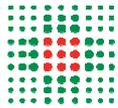
cancers [Herschkowitz JI et al. 2008]. Rb functional inactivation may result also from cyclin D1 overexpression or p16INK4A inactivation, and is frequent in breast cancer, as shown by studies of Rb-loss gene signatures identifying tumors with deregulated Rb [Ertel A et al. 2010, Herschkowitz JI et al. 2008].

CDKN2A is deleted in 3-8% of breast cancers, more frequently in the triple-negative subtype [Johnson J et al. 2016, Cairns P et al. 1995], and may be mutated or silenced by promoter methylation [Ruas M and Peters G, 1998].

p21Cip1 expression is frequently reduced as a consequence of TP53 mutation [Musgrove EA et al. 1995] or MYC overexpression [Mukherjee S et al. 2005], and p27Kip1 expression is reduced as a result of HER2 amplification [Chu IM et al. 2008].

In summary [Cancer Genome Atlas Network 2012, Witkiewicz AK and Knudsen ES, 2014], in luminal breast cancers estrogen receptor (ER) signaling induces cyclin D1 transcription, and there is often, particularly in luminal B tumors, cyclin D1 overexpression or gene amplification and CDK4 gain; p16INK4A or Rb losses are quite rare in luminal A, and somewhat more frequent in luminal B tumors.

Aberrations of p16INK4A and those of Rb are mutually exclusive, as tumors with loss of p16INK4A have wild-type RB, while tumors with mutant RB show high expression of p16INK4A [Witkiewicz AK and Knudsen ES, 2014].

Studies on the relationship between Rb proficiency and response to chemotherapy have yielded conflicting results. In adult fibroblasts with conditional RB knockout, loss of RB disrupted the DNA damage checkpoint elicited by either cisplatin or camptothecin exposure, abrogating cell cycle arrest and leading to accumulation of DNA double-strand breaks [Bosco EE 2004]. RB knockdown in breast cancer cell lines and xenograft models, resulting in E2F target gene deregulation, prevented cell-cycle inhibition following cisplatin and tamoxifen, and led to increased sensitivity to cisplatin but resistance to tamoxifen [Bosco EE 2007a, Bosco EE 2007b]. Deregulation of the Rb pathway, defined through an RB gene expression signature in tumor specimens from breast cancer patients, was associated with early disease recurrence after adjuvant tamoxifen [Bosco EE 2007a, Bosco EE 2007b]. Absence of RB expression was associated with better outcome in patients treated with adjuvant cyclophosphamide, methotrexate, and 5-FU, and with poorer outcome after
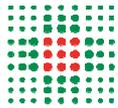
adjuvant tamoxifen, and 5-FU and methotrexate were shown to inhibit the growth of RB1-silenced but not of Rb proficient breast cancer cell lines [Derenzini M et al, 2008]. An RB-loss signature is associated with improved response to chemotherapy and longer relapse-free survival in patients with ER-negative breast cancers [Ertel A, 2010], and immunohistochemically detected Rb loss in triple-negative breast cancer correlates with benefit from adjuvant chemotherapy [Treré D et al. 2009]. On the other hand, RB mutations or delations have been found to be significantly associated with primary resistance to treatment with either 5-FU/mitomycin or doxorubicin in locally advanced breast tumors [Berge EO 2010], and a non-significant association with resistance to anthracyclines has been observed in patients with metastatic breast cancer [Berge EO, 2011].

In order to address these discrepancies, some studies have explored the combined contribution of the Rb status and p53 status in mediating response to chemotherapeutic agents [Zhu L et al, 2015]. In a retrospective study, p53 status, evaluated by immunohistochemistry, was found to have no predictive value for benefit from adjuvant cyclophosphamide, methotrexate and fluorouracil when considered independently of Rb, but was significantly associated with prognosis in patients with cancers with functioning Rb [Derenzini M, 2009]. In a retrospective study on patients treated with doxorubicin or fluorouracil + mitomycin for locally advanced breast cancer, genetic alterations leading to concomitant inactivation of both the p53 and the Rb pathways predicted resistance towards chemotherapy more strongly than inactivation of either pathway alone [Knappskog S, 2015].

The Cyclin D – CDK4/6 – Rb pathway is the target of CDK4/6 inhibitors. The expression of a functioning Rb protein is required for CDK4/6 inhibitors activity [Finn RS, BCR 2009], but loss of Rb function may occur in breast cancers, due to gene mutations or deletion and more frequently due to excessive phosphorylation resulting from activation of upstream pathways or loss of inhibitors. The identification of other response predictors has been elusive until now. Loss of p16 function, due to gene (CDKN2A) mutations or loss, as well as cyclin D1 gene (CCND1) amplification, expected to portend responsiveness from preclinical data [Finn RS, BCR 2009], were not associated with activity in clinical trials [Finn RS, Lancet Oncol 2015]. Predictors of CDK4/6 inhibitors sensitivity have been studied by Gong and colleagues with an antiproliferative screening on a large panel of cancer cell lines, using primarily the CellTiterGlo assay (an ATP-based measure of cell viability)

[Gong X, 2017]. CDKN2A loss or mutations confer only intermediate sensitivity to CDK4/6 inhibitors. High levels of cyclins D (D1, 2, or 3) expression are usually present in cancer cell lines responsive to CDK4/6 inhibitors but are not sufficient to confer sensitivity. Several genetic aberrations that lead to activation of D-type cyclins, referred to by the authors as "D-cyclin activating features" (DCAF), have been found to be associated to CDK4/6 inhibitors sensitivity in different cancer cell lines, including CCND1 translocation, CCND1-3 3'UTR loss (leading to a truncated CCND transcript with increased mRNA stability), CCND2 or CCND3 amplification, Kaposi sarcoma virus D-type cyclin (K-cyclin) loss and FBXO31 loss [Gong X, 2017]. CCND1 amplification is associated with high sensitivity to CDK4/6 inhibitors in ER-positive breast cancer cell lines, as well as in thyroid cancer cells, but with relative insensitivity in other cancer cell types (perhaps because of the preminent effect of neighbouring oncogenes co-amplified). Transcriptome data confirm the positive association of CCND2 and CCND3 mRNA levels with sensitivity to CDK4/6 inhibitors, while levels of CCND1 mRNA show inverse correlation with sensitivity. Protein data show that the expression levels of Rb or phosphorylated Rb positively correlate with sensitivity to CDK4/6 inhibitors, while levels of cyclin D1 are associated with relative insensitivity and p16 protein levels are not associated with sensitivity or resistance.

When using the CyQuant assay (a DNA content-based assay and a measure of DNA replication), results usually paralleled those from the CellTiterGlo assay in most cancer cell lines, apart from ER-positive breast cancer lines, that often showed a replication block also in presence of cell viability, highlighting the limited capacity of CDK4/6 inhibitors to completely suppress metabolism and growth. Fulvestrant synergizes with CDK4/6 inhibitors leading to complete metabolic suppression in ER-positive breast cancer cell lines.

Inhibition of CDK4/6 in CDKN2A mutant cells leads, after a transient suppression of Rb phosphorylation, to an increase in CDK2 activity, responsible for the reactivation of Rb phosphorylation and resistance to CDK4/6 inhibitors treatment. This fits with the finding that amplification of CCNE1 (coding for cyclyn E) predicts resistance to CDK4/6 inhibitors. On the contrary, sensitive cells show persistent suppression of Rb phosphorylation and lack of CDK2 activation after exposure to CDK4/6 inhibitors, resulting in cell senescence and apoptosis.

CDK4/6 kinases also phosphorylate factors involved in functions other than cell cycle regulation, affecting cell differentiation, mitochondrial activity, metabolism, antigen processing and presentation and immunogenicity [Klein ME, 2018]. Depending on cell type and transforming event, Rb-proficient cells can undergo quiescence or senescence or apoptosis in response to CDK4/6 inhibitors, but determinants of this different fates are not known. CDK4/6 inhibitors induce oxidative stress with ROS production in ER-positive breast cancer cells, which activate autophagy as a stress response mechanism, and autophagy degrades ROS preventing the induction of senescence. Combining CDK4/6 inhibitors with autophagy inhibitors, such as chloroquine, hydroxychloroquine, and others, induces senescence [Vijayaraghavan S, 2017]. In this study the expression of Rb protein was necessary for responsiveness to CDK4/6 inhibitors, while the expression of a low-molecular weight cyclin E isoform (LMWE), resulting from post-translational cleavage of full-length cyclin E, significantly reduced sensitivity to palbociclib. This highlights the importance of an intact G1/S transition for response to CDK4/6 inhibitors. The predictive value of Rb and LMWE expressions were confirmed on tumor samples from patients treated with fulvestrant + palbociclib for advanced breast cancer, showing better PFS for Rb+/LMWE- patients, intermediate for Rb+/LMWE+ and shorter PFS for Rb-/LMWE+ patients.

Several gene expression signatures associated with inactivation of the Rb pathway have been developed [Ertel A, Cell Cycle 2010; Chicas A, Cancer Cell 2010; Lara MF, Mol Carcinog 2008; Markey MP, Cancer Res 2002; Markey MP, Oncogene 2007; Witkiewicz AK, Clin Cancer Res 2012; Herschkowitz JI, Breast Cancer Res 2008], and one of them has been shown to discriminate between palbociclib-resistant and sensitive breast cancer cell lines [Malorni L, 2016]. If validated within a clinical trial this could become a further useful biomarker. Finally, serum levels of thymidine kinase, a key enzyme in DNA synthesis with peak expression in the S phase of cell cycle, have been shown to represent a pharmacodynamic marker of CDK4/6 inhibition in patients treated with neoadjuvant palbociclib [Bagegni N, Breast Cancer Res 2017].

p38 and JNK MAPK pathways

The Mitogen Activated Protein Kinase (MAPK) cascades form signal transduction pathways that respond to various extracellular stimuli and regulate several cellular processes, including
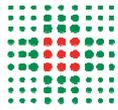
proliferation, differentiation, motility, stress response, survival, autophagy and apoptosis [Wagner EF et al, 2009; Plotnikov A et al, 2011]. They show a typical three-tier architecture, consisting of three core kinases (MAP3K, MAPKK, and MAPK), with some additional upstream (MAP4K) and downstream (MAPKAPK) components. The sequential activating phosphorylation of the kinases forming a cascade leads to the activation of target regulatory proteins represented by transcription factors and chromatin remodeling proteins, which mediate the cellular effects. At least four different MAPK cascades have been identified in mammals: extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38, and ERK5. While the ERK1/2 cascade is mainly activated by mitogenic stimuli via membrane receptor tyrosine kinases, the JNK and the p38 cascades are activated by stress stimuli, including cytotoxic drugs, ionizing radiations, heat and cold shocks, oxidative damage producing reactive oxygen species. They are therefore also called stress-activated protein kinase (SAPK) cascades, although they can be activated also by other stimuli, including mitogens.

There are three JNK genes in humans: JNK1, JNK2, and JNK3, each translated in 2-4 isoforms through alternative splicing. Activation of the JNK cascade by stress and other stimuli is mediated by small GTPases such as CDC42 and Rac1, or by adaptor proteins like TRAF. They can activate different types of MAP4Ks, or directly different types of MAP3Ks, which lead to activation of MKK4 and MKK7, which in turn activate the JNKs. JNKs, either directly or through putative MAPKAPKs like MST1 and MAPKAPK3, phosphorylate a large number of substrates, mostly in the nucleus but also in the cytoplasm, mediating cellular processes among which apoptosis but also autophagy.

There are four genes encoding p38 in humans: α, β, γ, and δ, p38α being expressed in most tissues and the others being more tissue-specific. The activation of the p38 cascade by stress-related and other stimuli occurs via small GTPases, adaptor proteins, MAP4Ks and MAP3Ks, leading to activation of MKK3 and MKK6 (and sometimes MKK4), which in turn activate the p38s. These act through several MAPKAPK, including MAPKAPK2-5, MNK1/2, and MSK1/2, which activate their target molecules regulating stress response, cell cycle, apoptosis, senescence, and cell survival. Contrary to other MAPKs, the activation of p38 may also occur by autophosphorylation, stimulated by interaction with other molecules such as the adaptor protein Tab1, ZAP-70, or lipidic phosphatidyl inositol analogues.
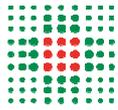
Considerable cross-talk exists between the JNK and the p38 cascades, whose differential activation depends on specific scaffold proteins, compartmentalization, and the substrates present.

Both the p38 and the JNK cascades are involved in the response to cytotoxic drugs and genotoxic stress [Olson JM et al, 2004; Sui X, et al. 2014], and can mediate both pro-death and pro-survival effects, depending on the cell type and strength of stimuli.

p38 activation has been shown to mediate the induction of apoptosis by cyclophosphamide [Pang H et al, 2011], cisplatin [Lou X et al, 2009], oxaliplatin [Chiu SJ et al, 2008], 5-fluorouracil [de la Cruz-Morcillo MA et al, 2012]. In other circumstances, p38 can also mediate resistance to cytotoxic drugs, including cisplatin [Chen SF et al, 2012], docetaxel [Gan L et al, 2011] and irinotecan [Paillas S et al, 2012], as well as to tamoxifen [Gutierrez MC et al, 2005]. p38-mediated resistance is attributed to the p38 role in regulating the balance between apoptosis and autophagy [de la Cruz-Morcillo MA et al, 2012; Paillas S et al, 2012], the latter being responsible for resistance by promoting cell survival. On the other hand, a prolonged autophagic response may induce autophagic cell death (type II programmed cell death) [Chiacchiera F et al, 2009].

Activation of the JNK cascade has also been shown to be involved in apoptosis induced by cytotoxic drugs such as mitoxantrone [Li Y et al, 2012], docetaxel [Zhu B et al, 2013], paclitaxel [Shajahan AN et al, 2012], and cisplatin [Mansouri A et al, 2003]. JNK is involved in autophagy induced for instance by bortezomib [Li C et al, 2012] and topotecan [Li DD et al, 2009], and while short activation of JNK promotes cell survival via autophagy, prolonged activation leads to autophagic cell death [Wei Y et al, 2008; Shimizu S et al, 2010]. Experimental and dynamic mathematical models of the JNK network, derived from cell lines and zebrafish models of neuroblastoma, showed that a switch-like JNK activation (favored by a positive feedback loop from JNK to MKK7) induces apoptosis, while the inability to initiate a switch-like JNK activation dampens apoptosis, and this differential behavior was associated with survival in a cohort of patients with neuroblastoma [Fey D et al, 2015].

Estrogen pathway and mechanisms of endocrine resistance

Estrogens regulate gene transcription in target tissues via the two estrogen receptors (ERs) subtypes α and β, members of the nuclear receptor superfamily [Thomas C et al, 2011; Nardone A
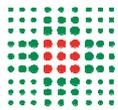
et al, 2015], encoded by the ESR1 and ESR2 genes, respectively. ERs α and β have opposite effects on breast cancer cells, the former stimulating proliferation and survival, and the latter inhibiting proliferation and invasiveness [Omoto Y et al, 2015], although the role of ERβ is still partly controversial.

ER have different known mechanisms of action [Hewitt SC et al, 2016; Chan HJ et al, 2015], some ligand-dependent, among which the classic genomic action and non-genomic actions involving the activation by estrogens of membrane G protein coupled ER (GPER, also known as GPR30), some ligand-independent, involving cross-talk with membrane growth factor receptors.

Both ER itself and many of the molecules involved in ER function and ER pathways have been implicated in endocrine resistance. This is often distinguished in "intrinsic" or "acquired", but their mechanisms are likely at least partly overlapping. Resistance to the main different classes of endocrine agents, selective ER modulators (SERMs), selective ER downregulators (SERDs), and aromatase inhibitors (AI) may, on the other hand, be at least partially based on different mechanisms [Clarke R et al, 2015; Ma CX et al, 2015].

Among the most important processes involved in endocrine resistance are the following:

- Loss of ER expression is found in a fraction of recurrent cancers [Kuukasjärvi T et al, 1996], and may be due to epigenetic silencing through ER promoter methylation [Sharma D et al, 2006], or to ER downregulation by proteasomal degradation [Pan X et al, 2011].

- ESR1 genetic alterations include [Jeselsohn R et al, 2015; Thomas C et al, 2015]:

- ESR1 amplification, whose impact in breast cancer is still controversial [Iggo RD et al, 2013]

- ESR1 genomic rearrangements, resulting in transcriptional dysregulation or in the generation of fusion proteins [Veeraraghavan J et al, 2014; Li S et al, 2013].

- ESR1 mutations, rare in primary breast cancer (about 0.5% in the updated The Cancer Genome Atlas project)[Memorial Sloan Kettering Cancer Centre. The cBioPortal for Cancer Genomics], but reported in a substantial fraction of patients with metastatic breast cancer progressing after endocrine therapy, particularly after treatment with aromatase inhibitors. These missense point mutations are clustered within the ER ligand-binding domain, and are most often gain of function mutations, leading to constitutive, ligand-independent ER activity, and are potentially treatable with SERDs like fulvestrant [Zhang QX et al, 1997; White R et al, 1997;
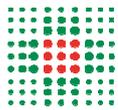
Lazennec G et al, 1997; Carlson KE et al, 1997; Robinson DR et al, 2013; Toy W et al, 2013; Li S et al, 2013; Schiavon G et al, 2015; Guttery DS et al, 2015; Chandarlapaty S et al, 2015].

- Hyperactivation of growth factor receptors pathways, including EGFR, HER2, IGFIR, and fibroblast growth factor receptor 1 (FGFR1), and their downstream signaling components, including MAPKs and the PI3K-Akt-mTOR pathways [Osborne CK et al, 2003; Kaufman B et al, 2009; Schwartzberg LS et al, 2010; Baselga J et al, 2012; D Paul et al, 2013; Osborne CK et al, 2011; Kaufman PA et al, 2010]

- Molecules of the PI3K-Akt-mTOR pathway are frequently altered in breast cancer, the α-catalytic subunit of PI3K (PIK3CA) being the most frequently mutated gene in breast cancer [The Cancer Genome Atlas, 2012]. Activation of PI3K phosphorylates the transcription factor FOXO3A, preventing its nuclear localization and induction of ESR1 transcription. Inhibition of PI3K may therefore induce the expression of ERs, and efficacy of PI3K inhibitors seems particularly important when combined with endocrine treatments [Crowder RJ et al, 2009].

- The cyclin D – CDK4/6 – RB pathway, activated by the ER and by different mitogenic and growth factor receptor signals, is frequently deregulated, particularly in luminal B breast cancer [The Cancer Genome Atlas, 2012]. Cyclin D1 (CCND1) amplification and CDK4 gains, as well as loss of the negative regulators CDKN2A (encoding p16) and CDKN2C (encoding p18), favor hormone-independent cell cycle progression, and activation of this axis is associated with endocrine resistance [Thangavel C et al, 2011; Finn RS et al, 2015; Turner NC et al, 2015].

- Cancer somatic genomic alterations, assessed by whole genome massive parallel sequencing on baseline tumor biopsies in patients receiving neoadjuvant aromatase inhibitors, highlighted potential predictors of response or resistance to these drugs [Ellis MJ et al, 2012; Gellert P et al, 2016]. Among 18 significantly mutated genes identified, mutant MAP3K1 was associated with luminal A subtype, low-grade histology and low proliferation rates, whereas mutant TP53 correlated with the luminal B subtype and high proliferation rates before and after treatment. GATA3 mutations correlated with greater suppression of proliferation after AI treatment, but not with baseline Ki67 levels, suggesting its potential as predictor of AI responsiveness. Pathway analysis through the PARADIGM tool showed that mutations in MAP2K4, a MAP3K1 substrate, were associated with luminal A features, whereas mutations in TP53, BIRC6,
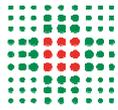
CDKN1B, RUNX1, and the long non-coding RNA MALAT1 were associated with luminal B features [Goldstein TC et al, 2013].

Circulating Tumor Cells (CTCs)

CTCs have been shown to have prognostic significance both in early and in advanced breast cancer [Banys-Paluchowski M et al, 2016]. CTCs can be found in 40 - 80% of patients with metastatic breast cancer. In this setting, a level of CTCs ≥5 cells/7.5 ml blood before initiation of first-line therapy is associated with poorer disease-free and overall survival [Cristofanilli M et al, 2004; Cristofanilli M et al, 2005]. This has been confirmed by several studies and by a meta-analysis [Bidard FC et al, 2014]. The pooled analysis shows that baseline CTC count as well as CTCs changes with therapy have independent prognostic effects on PFS and OS, beyond that of classical clinico-pathological variables. Furthermore, increases in CTC counts 3-5 weeks after start of treatment, adjusted for baseline CTC count, is associated with shorter PFS and OS, as is an increase in CTC counts after 6-8 weeks. CTC counts have prognostic value both when measured at the time of diagnosis of metastases and at each subsequent follow up time point during therapy for metastatic disease [Hayes DF et al, 2006]. CTCs measurement allows an earlier and more reproducible evaluation of response to therapy compared to traditional imaging methods, and shows stronger correlation with OS [Budd GT et al, 2006].

Biological characterization of CTCs has highlighted differences among the primary tumor, metastatic lesions and CTCs, in terms of expression of hormone receptors and/or HER2 [Pestrin M et al, 2009; Fehm T et al, 2010; Babayan A et al, 2013; Kalinsky K et al 2015; Wallwiener M et al, 2015]. CTCs are likely the most relevant tumor cell subpopulation for metastatic dissemination, and their characterization could therefore be more informative than biopsies of the primary tumor or of single metastatic lesions to guide treatment of metastatic disease [Pantel K et al, 2013]. There is some evidence that treatments targeted to the phenotype of CTCs may eliminate persistent tumor cells from the peripheral blood or the bone marrow [Bozionellou V et al, 2004; Bernhard H et al, 2008]. Studies on the prognostic relevance of specific CTCs phenotypes have yielded contradictory results [Hayashi N et al, 2012; Wallwiener M et al, 2015; Beije N et al, 2016].
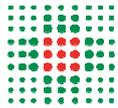
It is now possible to perform high throughput analyses on single CTCs, at the DNA, RNA, and protein level [Heitzer E et al, 2013; De Luca F et al, 2016], and this could help to inform treatment choices. Based on these premises, several clinical trials are ongoing, prospectively assessing the utility of CTCs as tools to monitor metastatic disease and guide therapy decisions.

8.2   Materials and methods

We will assess by *immunohistochemistry*, on **formalin fixed paraffin embedded tumor specimens** (taken from the metastatic sites when available, and from the primary tumor otherwise), the expression of the following biomarkers: phosphorylated ER, cyclin D1-**3**, cyclin E, low-molecular weight cyclin E isoform (LMWE), phosphorylated p38, JNK, p53, pRb, p16INK4a. A *gene expression profiling* study will be performed on formalin fixed, paraffin embedded samples from the primary tumor or from biopsies of metastatic sites (when available) in a subset of patients (responsive and resistant to CDK4/6 inhibitors and/or to chemotherapy). This will be done with the Nanostring nCounter using the specific breast cancer panel, or by RNA seq, and will include genes of the p53, Rb and p38/JNK pathways.

The number of **CTCs** will be measured (by means of the CELLSEARCH® CTC System) at baseline**,** after 6-8 weeks (2 treatment cycles) and at disease progression. CTC-positive samples will be processed by the micro-dissection microscope (MDM) system to recover the single CTCs and to develop the copy number variation (CNV) and mutation profile analysis for genes of the p53, Rb and p38/JNK pathways at single cell level. In a subgroup of patients, enrolled at Meldola, Forlì and Cesena hospitals, the biological features of CTCs will be assessed by means of the lab-on-a-chip system DEPArray NxT (Menarini, Silicon Biosystem), that combines the ability to manipulate and analyze single pure cells in fluorescence microscopy. This will allow to characterize the sub-phenotype expressed by CTCs and recover live single cells to evaluate the expression of epithelial markers (**e.g.** EpCAM, panCK, E-cadherin), mesenchymal markers (e.g. vimentin, N-cadherin, O-cadherin) and markers of stemness (e.g. ALDH1, CD44, ABCG2), as well as the expression profile of genes of the p53, Rb and p38/JNK pathways, by RNA-Sequencing analysis.
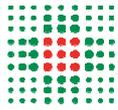
In further correlative studies we will assess by NGS, on **circulating cell-free DNA**, the mutational status and CNVs of genes of the p53 pathway, cell cycle/Rb pathway, p38/JNK MAPKs stress pathways, known to be with some frequency altered in breast cancer.

These will include the known genetic predictors: CCND1 translocation, CCND1-3 3'UTR loss, CCND2 or CCND3 amplification, Kaposi sarcoma virus D-type cyclin (K-cyclin) loss and FBXO31 loss.

Serum levels of **thymidine kinase 1 (TK1)** will be measured by ELISA at different time points during treatment.

Tumor tissue samples: an archived formalin-fixed paraffin-embedded (FFPE) block must be collected, from biopsy of a metastatic site when available, or from the primary tumor otherwise. If blocks are unavailable, provide 1 hematoxylin-eosin section and a total of 10 white sections of 5µM placed on positive charge slides from the primary/metastatic tumor sample.

Blood withdrawals:

- **Circulating DNA**: 10 ml of blood must be collected in a Vacutainer test tube with EDTA (CBC tubes with purple cap), at baseline and every 3 months until disease progression. Gently mix the blood collection tube by inverting 8-10 times immediately after collection. Then centrifuge immediately at 1500 to 2000 g for 15 minutes. This will get approximately 7.5 ml of plasma, and 2.5 ml of cellular pellet. Transfer the sovranatant plasma in aliquots of 1 ml in 2 ml cryovials, and the cellular pellet in aliquots of 1 ml in other cryovials. All cryovials must be stored at -80°C until shipment. Each tube and criovial must clearly indicate:
    – protocol name
    – date of sampling
    – patient personal data or code.

    Blood samples for circulating DNA must be withdrawn before those for CTCs.

- **Circulating Tumor Cells (CTCs) count (all patients)**: blood samples for the CELLSEARCH® CTCs Test must be collected at baseline, 6-8 weeks after the beginning of study treatment, and at disease progression.
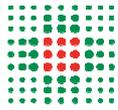
For specimen collection and preparation see Appendix F. For CTC count whole blood will be collected into a CellSave® Preservative Tube. Blood draw will be collected as follow:

- the Cell Save® Tubes will be drawn after other tubes, if multiple tube types are drawn for the protocol;

- the Cell Save® Tube will be filled until blood flow stops, to meet the required volume for the assays (CTC assay: at least 7.5 ml;

- the blood will be mixed IMMEDIATELY by gently inverting the CellSave® Tube 8 times.

- CellSave® Tubes will be labeled with patient ID, study number and date drawn.

Samples will be stored and transported at temperatures of 15°-30°C, protecting from extreme temperatures. All sample will be shipped to IOV within 36-72 hours after collection.

- **CTC single cell molecular analysis** (only patients enrolled at IRST): for RNA expression analysis on CTC, whole blood will be drawn into 10 ml EDTA tubes. For specimen collection and preparation see Appendix G.

The EDTA tubes will be filled until blood flow stops, and mixed IMMEDIATELY by gently inverting the EDTA Tube 8-10 times. Samples will be stored and transported at temperatures of 15°-30°C, protecting from extreme temperatures and shipped to IRST laboratory within 2-3 hours after collection.

8.3   Samples labelling and shipment

The blood samples for circulating DNA are shipped to the IRST IRCCS Biological laboratory in thermo-boxes containing sufficient dry ice. Dry ice will be provided by IRST IRCCS to each center, through the Dry ice company.

The CTC blood draws in EDTA tubes from Forlì and Cesena hospitals, are shipped to the IRST IRCCS Biological laboratory in thermo-boxes at room temperature.

The Tumor tissue samples are ambient shipped to the IRST IRCCS Biological laboratory.

The Shipment bill and the Shipper's account number for the Courier will be provided by IRST IRCCS.
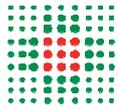
The shipment of samples must be announced via fax (+39 0543 739221) or via e-mail (daniele.calistri@irst.emr.it) including date of shipment and shipping bill number.

Every blood tube and/or slide with paraffin embedded tissue sections (please use special boxes for slides) will be labelled with the following information: Study code IRST174.19, site number, subject number, type of sample, timing and date of collection, in order to maintain rigorous confidentiality standards.

The samples will be sent to Bioscience Laboratory of IRST IRCCS through internal procedures for IRST IRCCS Centers and through courier for others Participating Centers (shipping paid by recipient), accompanied by the "Shipment Form", from Monday to Thursday to the following address:

<div align="center">

Dr. Daniele Calistri
c/o Laboratorio di Bioscienze,
IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST),
Via Maroncelli 40,
47014 Meldola (FC).
E-mail: daniele.calistri@irst.emr.it
Phone: +39 0543 739229, Fax: +39 0543 739221

</div>

After receiving the samples, the IRST IRCCS laboratory staff will complete the "Sample Arrival Form" (see specific guide lines), in order to maintain rigorous confidentiality standards.

CTC count will be assessed with the CELLSEARCH® CTCs Test at the Laboratory of Istituto Oncologico Veneto (Dr.ssa Rita Zamarchi).

The shipment of blood samples for CTCs count (Cell Save® Tubes) must be announced via e-mail (rita.zamarchi@unipd.it) including date of shipment and shipping bill number.

Every Cell Save® Tube will be labelled with the following information: Study code IRST174.19, site number, subject number, type of sample, timing and date of collection, in order to maintain rigorous confidentiality standards.

The samples will be sent to Laboratory of Istituto Oncologico Veneto through courier (shipping paid by recipient), accompanied by the "Shipment Form", from Monday to Thursday (8.30-15.00) to the following address:
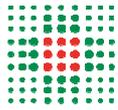
Dr. Rita Zamarchi
Laboratorio DME-CTC IOV
piano 7 - lato nord
Istituto di Ricerca Pediatrica Città della Speranza
Corso Stati Uniti 4 - 35129 Padova
E-mail: rita.zamarchi@unipd.it

8.4 Sample analysis, confidentiality and sample destruction

Genetic information obtained from patient samples will remain confidential. All patients evaluated for translational studies will be assigned an identification code (subject number) in order to maintain rigorous confidentiality standards.

The biomolecular characterization of biological samples will be performed at the Biological Laboratory of IRST IRCCS.

Samples will be destroyed after 15 years from the end of the study and all associated genetic data will be deleted from the study repository.

8.5 Data collection

Biological samples will be analyzed by IRST IRCCS biological laboratory according with its laboratory procedures.

9. MEASUREMENT OF EFFECT

9.1 Efficacy Parameters

Progression-Free Survival (PFS) is the time from the date of randomization to the date of the first observation of documented disease progression or death due to any cause. Patients without tumor progression at the time of analysis will be censored at their last date of tumor evaluation.

Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Time to Progression (TTP) is defined as the time from randomization (or registration) to
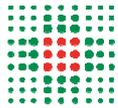
progression, or censored at date of last disease evaluation for those without progression reported.

9.2   Method and Timing

Response and progression in this study will be evaluated using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) Committee (see Appendix D). Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria.

Techniques used to measure disease should be the most accurate, reliable and reproducible methods that are routinely used.

9.3   Duration of response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

9.4   Response Review

Not applicable.

9.5   Other Response Parameters

See section 2 (objectives and outcome measures/endpoints)
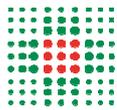
10. SAFETY REPORTING

Analyses will be performed for all patients having received at least one dose of study drug. CTCAE Version 5.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. If the experience is not covered in the modified criteria, the guidelines shown in the table below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

10.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical investigation participants administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.
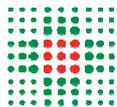
Adverse Reaction (AR): All untoward and unintended responses to a medicinal product related to any dose.

The phrase "responses to a medicinal products" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, e.g., the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Promoter as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

Severe Adverse Events: To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

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

Serious Adverse Event (SAE) or Serious Adverse Reaction:

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

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

A SAE or reaction is any untoward medical occurrence that at any dose:

- Results in death,
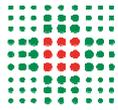
- Is life-threatening*,

NOTE: the term "life-threatening" in the definition of an SAE 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 that hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,

NOTE: the term "hospitalisationis" is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE.

- Results in persistent or significant disability/incapacity,

- Is a congenital anomaly/birth defect, or

- Is otherwise considered medically significant by the Investigator.

NOTE: medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Suspected Unexpected Serious Adverse Reactions (SUSAR): A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

AE Reporting Period

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.
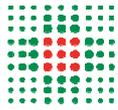
Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

Post-study AE

All unresolved AEs should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. The investigator should document in the eCRF and notify the study Promoter of any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

10.2 Reporting Procedures for All AEs

All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study medication, will be recorded on the eCRF.

The following information will be recorded: description, date of onset and end date, severity (according to CTCAE version 5.0), assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.
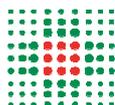
AEs considered related to the study medication as judged by a medically qualified investigator or the Promoter will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The relationship of AEs to the study medication will be assessed by a medically qualified investigator.

AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.
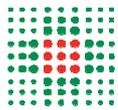
Any pregnancy that occurs during study participation must be recorded within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities or maternal and newborn complications.

10.3   Reporting Procedures for SAEs

The Investigator is responsible for reporting all SAE, related or not to the study treatment, occurring during the treatment period and within 30 days of the last protocol treatment, to the "Safety Desk". Any late Serious Adverse Drug Reaction (SADR), occurring after this 30-day period, should follow the same reporting procedure.

If a SAE occurs, the following action must be taken by the investigator:

Fill in the SAE form and send by fax within 24 hours of the initial observation of the event, to the Promoter:

<div align="center">

IRST Safety Desk
FAX 0543 739288
e-mail: fv.ct@irst.emr.it

</div>

- Attach a report of the event and a copy of all examinations that were carried out, including the dates on which these examinations were performed. For laboratory tests, normal laboratory ranges must also be included.

- All forms must be dated and signed by the responsible investigator or one of his/her authorized staff members.

- Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form and faxed to IRST.

- IRST Safety Desk will perform an initial check of the information and ensure that it is reviewed by the responsible safety physician.

- The IRST safety desk will send the SAE report to national authorities, Ethical Committees and investigators as appropiate, according to local regulations.
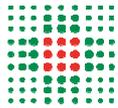
- IRST will report all SUSARs to the Competent Authorities and the Ethical Committees concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. IRST will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

- In addition to the expedited reporting above, the IRST Safety Desk shall submit once a year throughout the clinical trial or on request a safety report to the Competent Authority and Ethical Committees.

11. STATISTICAL CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

11.1 Study Design/Endpoints

This study is a multicenter, two-arm, open label group sequential response adaptive randomized phase 2 trial designed to compare the efficacy and safety of a combination of chemotherapy plus endocrine therapy (AI or fulvestrant) versus a cyclin-dependent kinase 4/6 inhibitor plus endocrine therapy (AI or fulvestrant) as treatment for patients with locally advanced or metastatic HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance.

11.2 Sample Size, Accrual Rate and Study Duration

The primary endpoint is PFS, and the underlying statistical hypothesis is that the combination of chemotherapy plus endocrine therapy (AI or fulvestrant) (i.e. arm B) will improve PFS compared with concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant).

Assuming that the survival times follow an exponential distribution parameterized in terms of its expected value, the statistical hypothesis system is $H_0: \theta_A = \theta_B$; $H_1: \theta_B > \theta_A$ at $\alpha = 0.10$ significance level.

A maximum sample size of 150 patients will be recruited in a 23 month period for an estimated accrual rate of around 6.5 patients per month; they will be monitored for a 16 month follow-up.

Initially, the patients will be randomly assigned according to block randomization (with blocks of size two) until two events (i.e. first disease progression or death whichever occurs first) are experienced in both arms. At this point, the allocation probabilities will be computed for the first time according to the Doubly-adaptive Biased Coin Design (DBCD) [Hu 2004] for time-to-event data [Zhang 2007] targeting the Neyman allocation rule (i.e. the one minimizing the total number of patients $n = n_A + n_B$). This randomized response adaptive procedure skews the allocation probabilities towards the arm with a longer estimated PFS allowing for a sensible ethical gain. Subsequently, these probabilities will be updated after 105 and 128 patients enter the study. At these time points, interim analyses will be performed in order to test the null hypothesis of equality of survival times [Zhu 2010]: whether will it be rejected the study is stopped and treatment B declared superior to A. In order to preserve the nominal significance level for the final analysis, Lan and DeMets α-spending function [Lan KG 1983] will be adopted for opportunely correcting the intermediate significance levels. The introduction of the interim analyses further increases the ethical component of the design ensuring that no more than necessary patients will be enrolled. Table 1 shows the results of Monte Carlo simulations carried out under a growing median PFS in arm B –i.e. $_{med}\theta_B$ ranges from eight to 14 months– against a fixed eight month PFS in arm A in order to evaluate the operating characteristics with a maximum sample size of 150 patients. Under the null hypothesis (first row of Table 1) both the usual completely randomized design (CR) and the group sequential DBCD (G-DBCD) show a good control of type-I error rates. Further simulations, not displayed here for sake of brevity, confirmed that both designs maintain this paramount capability even for higher sample sizes and/or longer trial recruitment and duration. Furthermore, G-DBCD expected sample size (ESS) is close to the 150 patients CR fixed one while a good amount of patients is adaptively allocated ($\tau = 0.779$) in an almost balanced

way ($\pi_B = 0.498$). This means that under the null hypothesis of equal PFSs, neither arm is preferred.

Increasing the median PFS of treatment B, allows to appreciate the considerable gain in terms of power induced by G-DBCD which allows the log-rank test to far better detect a longer PFS in arm B than under CR. Furthermore this ability is coupled with a progressively lower expected sample size compared to the fixed CR one. Less patients are adaptively assigned as the increase of $_{med}\theta_B$ results in a delay of the starting of the response adaptive randomization procedure since in average it takes more time to observe two events in arm B. On the other hand, the allocation proportion are increasingly skewed towards treatment B as it progressively shows a longer PFS.

Let us a now consider a median eight month PFS for arm A (Paloma 3 Study, supplementary material of Turner NC, NEJM 2018) and a 12 month one for arm B –i.e. $_{med}\theta_A = 8$ and $_{med}\theta_B = 12$. Under this more likely scenario, the log-rank test coupled with G-DBCD is able to detect a four month median PFS increment in arm B with a considerably higher simulated power than under CR design: 0.911 versus 0.717, respectively. In addition, when compared to the fixed 150 patient sample size of the CR design, the G-DBCD shows a reduction of almost 29 patients in the expected sample size (ESS) due to the introduction of interim analyses allowing for early stopping for efficacy. This means that on average around 19% less individuals will be enrolled in the trial, stressing the considerable ethical gain of the proposed strategy. Concurrently, for the log-rank test to achieve the same power (0.911) as under G-DBCD analytical results shows that under CR design on average it needs around 184 total patients –i.e. an increase of around 52% patients with respect to the 121.259 ESS. Finally, under G-DBCD, on average, slightly more than 56% of the ESS is allocated to arm B (π_B): a considerable ethical gain is again appreciable.

Table 1. Results of the Monte Carlo simulation for log-rank test coupled with the complete randomization (CR) and group sequential DBCD (G-DBCD) assuming a median PFS of eight months for arm A and an increasing one for the experimental one ($_{med}\boldsymbol{\theta_B}$): power of the two strategies and expected sample size (ESS), allocations proportions to the experimental ($\boldsymbol{\pi_B}$) and percentages of patients adaptively allocated to

arm B (τ) under G-DBCD.

$_{med}\theta_B$	power		ESS	π_B	τ
	CR	G-DBCD			
8.0	0.084	0.089	147.014	0.498	0.779
9.0	0.205	0.458	138.317	0.509	0.758
10.0	0.384	0.741	129.897	0.529	0.732
11.0	0.564	0.841	125.304	0.547	0.713
12.0	0.717	0.911	121.259	0.563	0.695
13.0	0.829	0.948	117.939	0.580	0.678
14.0	0.908	0.972	115.130	0.595	0.662

Figure 1. Power of the nonparametric test under complete randomization (CR) and group sequential DBCD (G-DBCD).

All patients fulfilling the eligibility criteria will be randomized by the Biostatistics and Clinical Trial Unit of the CC, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST). No blinding is planned.

The study duration will be 39 months; 23 months of accrual and 16 months of follow-up on the last participant enrolled.

11.3 Stratification Factors

No stratification factor will be considered in the primary endpoint analysis.

11.4 Analysis of Primary Endpoints

The Intention-to-treat (ITT) population is defined as the population of all enrolled patients. The activity (AP) and the safety population (SP) are considered as all patients who received, in each treatment group, at least one dose of treatment.

Descriptive statistics will be reported for patients and tumors characteristics. Proportions will be compared with chi-square or Fisher exact test as indicated, and continuous variables will be compared by t-test or non parametric tests according to data distributions, providing 95% confidence intervals (95% CI).

Time to event data will be analyzed using Kaplan-Meier curves, with 95% CI for median time and for each year of follow-up calculated with nonparametric methods.

Comparisons between the two treatment arms will be performed using the log rank test, at a significance level of 10%. Unadjusted and adjusted hazard ratios (HR) will be calculated using Cox proportional-hazard models, including or not the main know prognostic and predictive factors. Two-sided 95% CI for each HR will be provided.

11.5 Analysis of Secondary Endpoints

Clinical endpoints will be analyzed with the methods described in the primary endpoint section.

Time course of biomarkers levels and of quality of life scores will be compared between the two
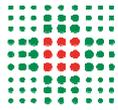
groups by mixed effects models for repeated longitudinal data.

11.6 Interim Analysis

Two interim analyses are planned after 105 and 128 patients are enrolled (i.e. after 70% and 85% of the maximum sample size, respectively). At each halt the PFS equality between the two arms against the superiority of arm B will be inferred by means of the log-rank test. The family wise error rate will be controlled by means of Lan and DeMets α-spending function [Lan KG 1983] in order to preserve the nominal $\alpha = 0.10$ significance level toward the final analysis.

11.7 Reporting and Exclusions

Evaluation of toxicity.

All patients will be evaluable for toxicity from the time of their first administration of chemotherapy

Evaluation of response.

Only those patients who have received at least one dose of therapy, and have had disease re-evaluated, will be considered evaluable for response. These patients will have response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

11.8 Procedure(s) to account for missing or spurious data

Missing data will be assumed to be missing completely at random for all analyses and no imputation will be done to estimate missing observations.

11.9 Other statistical considerations.

Not applicable.
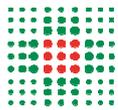
12.   ETHICAL ASPECTS

12.1   Declaration of Helsinki and ICH Guidelines for Good Clinical Practice

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004) and is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996, the Directive 2001/20/EEC of the European Parliament and other relevant local legislation and applicable regulatory requirements Subject data protection.

12.2   Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with the Declaration of Helsinki. The Investigator has responsibilities to the Health Authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms.

The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub investigators and other study staff members, adhere to the study protocol and all local and federal regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodic monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12.3   Independent Ethical Committee (IEC)

The protocol, informed consent and any accompanying material provided to the patient will be
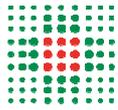
submitted by the investigator to an Independent Ethical Committee for review. Approval from the committee must be obtained before starting the study. Any modifications made to the protocol, informed consent or material provided to the patient after receipt of the Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements. The IEC approval report must contain details of the trial (title, protocol number and version), documents evaluated (protocol, informed consent material) and the date of the approval.

12.4   Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from each subject prior to entering the trial or, where relevant, prior to evaluating the subject's suitability for the study.The informed consent document used by the Investigator for obtaining the subject's informed consent must be reviewed and approved by the IEC.

All patients will be informed of the aims of the study, the possible AEs, the procedures and possible hazards to which he will be exposed, and the mechanism of treatment allocation. In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered for the study. This must be done in accordance with the national and local regulatory requirements.

The written ICF should be signed and personally dated by the patient or by the patient's legally acceptable representative. The clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study. A copy of the signed ICF must be provided to the patient or the patient's legally authorized representative. The orginal copy of the patient's signed written consent will be kept by the center
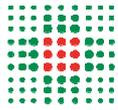
in the proper section of the Investigator Site File and must be available for verification by study monitors at any time.

12.5 Patient data protection

The ICF will incorporate wording that complies with relevant data protection and privacy legislation. In agreement with this wording, patients will authorize the collection, use and disclosure of their study data by the Investigator and by those persons who need that information for the purposes of the study.

The ICF will explain that the study data will be stored in a computer data base, maintaining confidentiality in accordance with national data legislation.

The ICF will also explain that for data verification purposes, authorized representatives of Promoter, a regulatory authority, an Ethics Committee may require direct access to parts of the hospital or practice records relevant to the study, including patients' medical history.

13. DATA COLLECTION

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Promoter (or designee), but will be identified by a site number, subject number.

eCRFs are to be completed through use of a Promoter-designated EDC system. Sites will receive training and have access to a manual for appropriate eCRF completion. eCRFs will be submitted electronically to the Promoter and should be handled in accordance with instructions from the Promoter. All eCRFs should be completed by designated, trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.
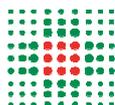
If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail.

At the end of the study, the investigator will receive patient data for his or her site in a readable format on a compact disc that must be kept with the study records. Acknowledgement of receipt of the compact disc is required.

14.   STUDY MONITORING

The Investigator agrees to to perform the study in accordance with ICH Good Clinical Practice.

The Investigator is required to ensure his compliance to the procedures required by the protocol with respect to the investigational drug schedule and visit schedule. The Investigator agrees to provide all information requested in the Case Report Form in an accurate and legible manner according to the instructions provided.

The Investigator has responsibilities to the Health Authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms.

14.1   Site Set-up and Initiation

All participating Investigators will be asked to sign the necessary agreements and supply a current CV to the CC or Promoter.

All members of the site research team will also be required to sign the "*Site Signature and Delegation Log"* .

Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, AE reporting, collection and

reporting of data and record keeping.

Sites will be provided with an Investigator Site File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The CC or Promoter
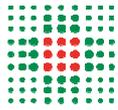
must be informed immediately of any change in the site research team.

14.2   On-site Monitoring

If a monitoring visit is required the CC, or Promoter will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the trial staff access to source documents as requested.

The main duty of the Trial Monitor is to help the Investigator and the Study Coordinators to maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

During each monitoring visit, the following points will be checked: subject informed consent, subject recruitment and follow-up, study drug allocation, subject compliance to the study treatment, study treatment accountability, AE documentation and reporting.

According to the guidelines on ICH Good Clinical Practice, the trial monitor will check the case report form entries against the source documents. This personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

14.3   Central Monitoring

Trials staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Trials staff will check data received for compliance with the protocol, data consistency, missing data and timing. Sites will be sent requests missing data or clarification of inconsistencies or discrepancies. For eCRF trials these requests may be generated by automated data validation checks.

14.4   Audit and Inspection

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

Sites are also requested to notify the CC or Promoter of any CA inspections.
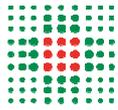
15. ADMINISTRATIVE REGULATIONS

The CC is responsible for drawing up the final version of the protocol, implementing the eCRFs, creating randomization lists and updating the electronic database, defining general organizational procedures, and organizing periodic meetings and newsletters. The CC will also undertake the following: support for the preparation of all documents needed for EC submission of the study protocol for each participating center, training of staff assigned to data collection, definition of monitoring procedures and monitor training.

15.1 Curriculum vitae

An updated copy of the curriculum vitae of each Principal Investigator, duly signed and dated, will be provided to the study monitor prior to the beginning of the study.

15.2 Secrecy agreement

All goods, materials, information (oral or written) and unpublished documentation provided to the Investigators, including this protocol and the case report forms, shall be considered confidential and may not given or disclosed to third parties.

15.3 Record retention in investigative centres

Study documentation includes all eCRFs, data correction forms or queries, source documents, Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms). Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug
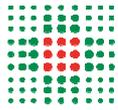
seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until 15 years after the completion and final study report of this investigational study.

15.4   Insurance

A clinical trial insurance has been arranged, according to the Italian law (DM of 14th of July 2009) for this specific trial. The clinical trial insurance is only valid if treatment is given in a center authorized by IRST IRCCS and which has obtained Ethical Committee approval. All daily clinical practice procedures refer to the business insurance of the participating center where the patient is treated.

16.   OWNERSHIP OF DATA AND USE OF THE STUDY RESULTS

The full ownership of the data generated in this study is retained by IRST and by all the investigators actively recruiting patients.

Data deriving from this clinical trial are not intended for drug registration or for patent applications, but only for scientific and educational purposes, which include presentation at scientific meetings, congresses and symposia and/or publication in scientific journals.

17.   PUBLICATION POLICY

Publications regarding the main study end-points will be prepared by the Chief Investigator. Authorship will be proportional to the accrual of each center. All the members of the steering committee and components of the CdC will be included in the authors list and all the investigators recruiting will be mentioned as contributors. Other area-specific publications will be prepared by the coordinators of the single treatment modalities to increase the visibility of the study and investigators. However, the publication of secondary endpoints is discouraged before publication
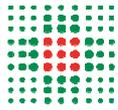
of the main endpoint and should be anyway discussed with the study and writing committee coordinators.

18. PROTOCOL AMENDMENTS

It is specified that the appendices, attached to this protocol and referred to in the main text of this protocol, form an integral part of the protocol.

No changes or amendments to this protocol may be made by the Investigators after the protocol has been agreed to and signed by both parties .Any change agreed upon will be recorded in writing, the written amendment will be signed by the Chief Investigator and by the Principal Investigator and the signed amendment will be appended to this protocol.

Approval / advice of amendments by Ethical Committees or similar body is required prior to their implementation, unless there are overriding safety reasons.

If the change or deviation increases risk to the study population, or adversely affects the validity of the clinical investigation or the subject's rights, full approval / advice must be obtained prior to implementation. For changes that do not involve increased risk or affect the validity of the investigation or the subject's rights, approval / advice may be obtained by expedited review, where applicable.

In some instances, an amendment may require a change to a consent form. The Investigator must receive approval / advice of the revised consent form prior to implementation of the change.
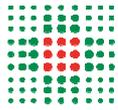
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APPENDIX A Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.
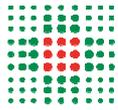
APPENDIX B NCI Common Termonology Criteria for AE

The manual is available here:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_

Reference_8.5x11.pdf
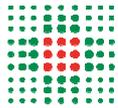
APPENDIX C Schedule of procedures

Time	Baseline	Cycle 1	Cycle 2	Cycle 3	Cycle n	End of treatment	Follow up visits[18]
Day	-28 to 0	1	1	1	1		
Informed consent	X						
Demographics[1]	X						
HER2 and ER/PgR status[2]	X						
Medical history and prior anticancer therapy[3]	X						
Current medical conditions and concomitant medications[4]	X		X	X	X	X	
Physical examination	X		X	X	X	X	
Vital signs[5]	X		X	X	X	X	
ECOG-PS	X		X	X	X	X	
CBC and serum chemistry[6]	X		X	X	X	X	
Coagulation tests[7]	V						
Urinalysis	V					V	
Pregnancy test[9]	X						
Cardiological examination[10]	V					V	
Formalin-fixed, paraffin-embedded tumor specimen[11]				X			
Blood samples for CTCs[12]	X			X		X	
Blood samples for circulating markers (other than CTCs)	X		Every 3 months (±8 days)			X	
Quality of life Questionnaires[13]	X		Every 3 months (±8 days)			X	
Serum CA15/3	X		Every 3 months (±8 days)			X	
Tumor evaluation[14]	X		Every 3 months (±8 days)				
Bone scan[15]	V						
LHRH analog[16]				X			
Treatment Compliance check			X	X	X	X	
Adverse events[17]	X	X	X	X	X	X	
Survival status							X
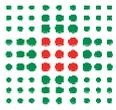
Treatment administration should begin ≤72 hours of the date of randomization.

1. The date of birth, gender and race will be recorded.
2. Includes confirmation and documentation of HER2 and ER/PgR status on the most recent tumor tissue.
3. Details of any history of prior or concurrent diseases or surgical interventions (including prior cancer therapies) will be recorded.
4. Clinical conditions, including assessment of symptoms and any residual toxicity relating to prior treatment (if applicable).
5. Blood pressure, pulse, body temperature, height and weight.
6. Complete blood count (CBC) with differential and platelet count; blood chemistry assessment including renal and liver function tests (e.g. serum creatinine, potassium, sodium, chloride, calcium, ALAT/SGPT, total bilirubin, alkaline phosphatase, total proteins and albumin).
7. Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR).
8. Serum or urine pregnancy test for women of child-bearing potential.
9. 12-lead electrocardiogram (ECG), cardiological examination and/or cardiac function assessment by ECHO or MUGA if clinically indicated (recommended for cardiotoxic drugs).
10. From primary tumor or, when available, from a metastatic biopsy performed before study treatment start.
11. Samples to be collected at baseline, 6-8 weeks after the beginning of study treatment, and 2-4 weeks after the last dose of chemotherapy.
12. EORTC QLQ-C30 Version 3.0, QLQ-BR23.
13. CT scan of the chest, abdomen and pelvis with contrast medium (CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis). In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted. Physical examination, with description (and measurement of the main diameter when feasible) of superficial tumor lesions (or picture if deemed useful).
14. If clinically indicated (recommended if bone pain is present or bone lesions are found at CT).
15. For premenopausal women, or men receiving LHRH analog, the treatment with LHRH analog can initiate during screening phase.
16. Assessment of adverse event (CTCAE version 5.0, Appendix B).
17. Every 3 months.

X: mandatory
V: if clinically indicated
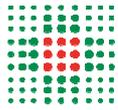
APPENDIX D Response Criteria

The revised RECIST guidelines (version 1.1) are available here:

http://ctep.cancer.gov/protocolDevelopment/docs/recist_guideline.pdf
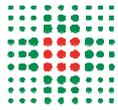
APPENDIX E World Medical Association Declaration of Helsinki

The current Declaration of Helsinki can be found on the World Medical Association web page via the link provided below:

http://www.wma.net
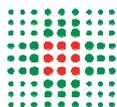
APPENDIX F Procedures for CTCs Specimen Collection and Preparation

PROCEDURA PER L'ESECUZIONE DEL PRELIEVO PER IL CONTEGGIO DELLE CELLULE TUMORALI CIRCOLANTI (CTCs)

L'analisi consente la numerazione delle CTC di origine epiteliale (CD45-, EpCAM+ e CITOCHERATINE 8, 18 e/o19+) presenti nel sangue intero.

Modalità di esecuzione del prelievo di sangue

Per la conta delle CTCs il prelievo di sangue intero (10 ml) deve essere necessariamente eseguito in **provette CellSave® Preservative Tubes**.

1. Il campione iniziale deve essere raccolto prima di avviare un regime terapeutico. I campioni successivi devono essere prelevati secondo il calendario previsto per i due bracci di studio
2. Il prelievo di sangue intero deve essere eseguito in modo asettico mediante venipuntura o porta venosa esclusivamente in provette CellSave® Preservative Tubes.
3. E' necessario riempire la provetta fino all'arresto del flusso sanguigno, per assicurare il corretto rapporto tra campione e mix di anticoagulante e conservante. E' importante miscelare immediatamente capovolgendo delicatamente la provetta otto volte. Il capovolgimento della provetta impedisce la formazione di coaguli. Una miscelazione effettuata in modo inadeguato o ritardato può portare a risultati errati.
4. I campioni raccolti devono pervenire al laboratorio **entro 36-72 ore** dal prelievo. Possono essere conservati e trasportati a temperatura ambiente (da 15 a 30°C). Non refrigerare.

E' bene preavvisare per e-mail (rita.zamarchi@unipd.it) la spedizione dei campioni. I campioni del venerdì devono arrivare entro le ore 10.00 del mattino, per poter essere processati in giornata.

I campioni vanno inviati (orario accettazione: lun-gio, 8.30-15.00) a:

Laboratorio DME-CTC IOV
piano 7 - lato nord
Istituto di Ricerca Pediatrica Città della Speranza
Corso Stati Uniti 4 - 35129 Padova
All'attenzione della Dott.ssa Rita Zamarchi
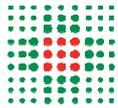
APPENDIX G Procedures for CTCs in EDTA tube; Specimen Collection and Preparation

PROCEDURA PER L'ESECUZIONE DEL PRELIEVO PER L'ARRICCHIMENTO DELLE CELLULE TUMORALI CIRCOLANTI (CTCs) VITALI E PER L'ANALISI DI ESPRESSIONE GENICA SU SINGOLA CELLULA

L'analisi consente la caratterizzazione delle CTC presenti nel sangue intero.

Modalità di esecuzione del prelievo di sangue

Il prelievo di sangue intero (10 ml) deve essere necessariamente eseguito in **provette EDTA Tubes**.

1. Il campione iniziale deve essere raccolto prima di avviare un regime terapeutico. I campioni successivi devono essere prelevati secondo il calendario previsto per i due bracci di studio.
2. Il prelievo di sangue intero deve essere eseguito in modo asettico mediante venipuntura o porta venosa esclusivamente in provette EDTA Tubes da 9-10 ml.
3. E' necessario riempire la provetta fino all'arresto del flusso sanguigno, per assicurare il corretto rapporto tra campione e mix di anticoagulante e conservante. E' importante miscelare immediatamente capovolgendo delicatamente la provetta otto volte. Il capovolgimento della provetta impedisce la formazione di coaguli. Una miscelazione effettuata in modo inadeguato o ritardato può portare a risultati errati.
4. I campioni raccolti devono pervenire al laboratorio **entro 3-4 ore** MAX dal prelievo. Possono essere conservati e trasportati a temperatura ambiente (da 15 a 30°C). Non refrigerare.

E' bene preavvisare per e-mail (massimiliano.bonafe@irst.emr.it) la spedizione dei campioni.
I campioni vanno inviati (orario accettazione: lun-ven, 8.30-15.00) a:

Prof. Massimiliano Bonafè
c/o Laboratorio di Bioscienze,
IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST),
Via Maroncelli 40,
47014 Meldola (FC).
E-mail: massimiliano.bonafe@irst.emr.it
Phone: +39 0543 739905, Fax: +39 0543 739221
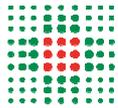
APPENDIX H Protocol Changes

Reason for changes

The protocol was amended following changes in clinical practice with the advent of new therapeutic options for hormone receptor-positive, HER2-negative metastatic breast cancer (also called "luminal" breast cancer).

The original study included a randomized comparison between concomitant chemo-endocrine therapy and chemotherapy followed by endocrine therapy as first-line treatment of luminal metastatic breast cancer with aggressive features, such as low expression of progestin receptors and/or high proliferative index (luminal B subtype).

When the project was presented there were no molecular targeted drugs registered for this breast cancer subtype and the therapeutic options were endocrine therapy and chemotherapy. It was therefore important to understand whether the concomitant administration of these two treatments (concomitant chemo-endocrine therapy) was more effective than their sequential administration (commonly used in the clinic and considered the "standard" arm of the study).

Recently, a new class of drugs, the cyclin-dependent kinase inhibitors 4 and 6 (CDK4/6 inhibitors) has become available in clinical practice. Given in combination with hormonal drugs, CDK4/6 inhibitors have led to a marked improvement in progression-free survival of patients with metastatic luminal tumors compared to what can be achieved with hormone therapy alone, both in the first treatment line (in combination with an aromatase inhibitor) and in the second line (in combination with fulvestrant). The combination of CDK4/6 inhibitors and hormone therapy also produce objective response rates comparable to those obtained with chemotherapy. These combinations have become standard treatment options in the first and/or second line of hormone therapy.

Therefore, today we cannot ignore the existence of a new "standard" therapy option in metastatic luminal carcinoma: combination therapy with a CDK4/6 inhibitor + an endocrine agent.

It was thus necessary to amend this study, proposing what is currently the most relevant comparison: that between CDK4 / 6 inhibitor + endocrine agent on one side (arm A) and chemotherapy + endocrine therapy on the other (arm B).

The study maintains the pragmatic character it originally had, leaving the clinician the choice of the CDK4/6 inhibitor drug (to be used according to the indications registered at the time of treatment), of the chemotherapy regimen (to be chosen among those considered standard for this breast cancer subtype and treatment line) and of the hormonal agent. The latter will be administered in arm A from the beginning of treatment with CDK4/6 inhibitor, while in arm B it will be at the discretion of the clinician to administer it concomitantly (from the beginning of chemotherapy: concomitant chemo-endocrine therapy) or sequentially (as maintenance therapy, after stopping chemotherapy at the end of 4-6 months of chemotherapy treatment: sequential chemo-endocrine therapy).

It was also decided to use the most efficient study design with group sequential adaptive randomization, moving from a phase 3 to a randomized phase 2 study but conserving adequate power for a comparison of treatment efficacy and introducing the possibility of early termination

of the study for efficacy, reducing the expected total number of patients and including more patients in the more effective treatment arm.

The biological part of the study was also appropriately modified to identify specific predictors of response to CDK4/6 inhibitors + endocrine therapy and those to chemo-endocrine therapy.

1) Title, page 1

Original text

Randomized clinical trial of concomitant chemoendocrine therapy versus chemotherapy followed by endocrine therapy as first line treatment of luminal B metastatic breast cancer.

Short title / Acronym: CHemo-ENDOcrine therapy in advanced breast cancer / CHENDO

Amended text

~~Randomized clinical trial of concomitant chemoendocrine therapy versus chemotherapy followed by endocrine therapy as first line treatment of luminal B metastatic breast cancer.~~

Group sequential response adaptive randomized clinical trial of chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer

Short title / Acronym: ~~CHemo-ENDOcrine therapy in advanced breast cancer / CHENDO~~
CDK4/6-inhibitor or chemotherapy, in combination with ENDOcrine therapy, for advanced breast cancer / KENDO

2) KEY TRIAL CONTACTS, page 2

Original text

Chief Investigator	Andrea Rocca Breast Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS …
…	…
Statistician	Oriana Nanni Unità di Biostatistica e Sperimentazioni Cliniche IRST IRCCS Via P. Maroncelli 40, 47014 Meldola (FC) - Italy phone: +390543739262 e-mail address: oriana.nanni@irst.emr.it

Amended text

Chief Investigator	Andrea Rocca Breast **Cancer** Unit

	Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS …
…	…
Statistician	**Alessandro Vagheggini** ~~Oriana Nanni~~ Unità di Biostatistica e Sperimentazioni Cliniche IRST IRCCS Via P. Maroncelli 40, 47014 Meldola (FC) - Italy phone: +390543739262 e-mail address: ~~oriana.nanni~~**alessandro.vagheggini**@irst.emr.it

3) PROTOCOL SIGNATURE PAGE, page 3

Original text

…

Oriana Nanni
Trial Statistician   Signature   Date

Amended text

…

Alessandro Vagheggini~~Oriana Nanni~~
Trial Statistician   Signature   Date

4) SUMMARY, page 4

Original text

Title	Randomized clinical trial of concomitant chemoendocrine therapy versus chemotherapy followed by endocrine therapy as first line treatment of luminal B metastatic breast cancer.
Short Title/ Acronym	CHemo-ENDOcrine therapy in advanced breast cancer / CHENDO
Protocol Code	IRST174.19 Identifier Code: L2P1388
Phase	Phase 3

Study Design		Prospective, open label, multicenter, randomized phase III study, comparing two strategies as first chemotherapy-based treatment for locally advanced or metastatic luminal breast cancer: - Arm A: concomitant chemotherapy plus an aromatase inhibitor (AI); the AI must continue until disease progression or toxicity or patient refusal; chemotherapy may be stopped after achievement of maximum response (generally after at least about 3-6 months of treatment) or in case of toxicity or patient refusal. - Arm B: chemotherapy followed by an AI at the time of progression to chemotherapy (if an endocrine therapy is deemed indicated by the treating physician) or as maintenance therapy after achieving maximum response to chemotherapy (generally after at least about 3-6 months of treatment) or after stopping chemotherapy for toxicity or patient refusal.
Back ground and Rationale for study		Although metastatic luminal breast cancer is most often treated with endocrine therapy as initial treatment, in some cases chemotherapy may be required, either at first metastatic relapse, or after failure of endocrine therapy. This applies particularly to the luminal B breast cancer subtype, characterized by expression of estrogen receptors but high proliferative index and/or low expression of progesterone receptors, whose prognosis is worse, and responsiveness to endocrine therapy poorer, compared with the luminal A counterpart. In some clinical conditions also luminal A breast cancer, characterized by expression of estrogen and progesterone receptors with low proliferative index, might require chemotherapy. In these cases, it remains unknown if the concomitant administration of an endocrine agent to chemotherapy might improve results compared to chemotherapy alone.
Timelines		Estimated duration for the main protocol: 4 years Study start (FPFV): 05/2017 Recruitment end (LPFV): 04/2019 Follow-up period end date (LPLV): 04/2020
Study Centers		Multi-center study
Objectives and outcome measures	Primary	To compare the efficacy of concomitant chemotherapy and AI versus chemotherapy followed by AI in terms of progression free survival (PFS).

	Secondary	To compare between treatment arms:
		• quality of life (EORTC QLQ-C30 and QLQ-BR23)
		• toxicity (CTCAE version 4.03)
		• time to treatment failure
		• time to progression to the therapeutic strategy
		• best response rate
		• duration of response
		• clinical benefit rate
		• overall survival (OS)
		• correlative biomarkers of response to chemotherapy and endocrine therapy:
		    – tissue markers (on the primary tumor and / or metastatic tissue)
		    – circulating markers (e.g. CTCs, ctDNA)

Number of Subjects		300 patients overall (150 patients in each treatment arm)
Diagnosis and Main Eligibility Criteria		Patient population: postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not) with HER2-negative, locally advanced or metastatic luminal breast cancer, candidate to their first chemotherapy-based treatment for advanced disease.

Inclusion criteria:

- Age ≥ 18 years.
- Histological diagnosis of HER2-negative luminal breast cancer (ER >10% of tumor cells), determined by local laboratory on most recent available tumor tissue.
- Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as "advanced breast cancer (ABC)").
- Candidate to chemotherapy-based treatment per the investigator best judgment; e.g. because of disease aggressiveness, short disease-free interval, elevated Ki67 [if available on a metastatic site], low expression of hormone receptors, extended visceral involvement, visceral involvement at risk for organ failure, uncontrolled symptoms), according to Associazione Italiana di Oncologia Medica (AIOM) Guidelines (2016 edition).
- Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not).
- Measurable disease according to RECIST 1.1 criteria, or not measurable but evaluable disease.
- No prior chemotherapy for advanced disease. Up to two prior lines of endocrine therapy for ABC, as well as targeted therapies (such as palbociclib and/or everolimus or investigative targeted therapies) administered as part of a prior hormonal regimen for ABC, are allowed.
- Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).
- Adequate organ (renal, hepatic, bone marrow, cardiac) functions.
- Female participants of child bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.
- Participant is willing and able to give informed consent for participation in the study.

Exclusion criteria:

- Any prior chemotherapy for advanced breast cancer
- Resistance to both non-steroidal and steroidal aromatase inhibitors, eg patients who progressed while on or within 12 months after the end of an aromatase inhibitor in the adjuvant setting and who progressed while on an aromatase inhibitor (of a different class) in the metastatic setting, or patients who progressed to both classes of aromatase inhibitors administered as two distinct lines of therapy for metastatic disease.
- Patients who have not recovered from adverse events due to prior therapies to grade ≤1 (excluding alopecia).
- Active central nervous system metastases.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to the chemotherapeutic or endocrine agents used in the study.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (patients with history of hepatitis B must undergo prophylactic therapy with lamivudine or other agent according to infectious disease consultation), symptomatic congestive heart failure, unstable angina pectoris, cardiac

	arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. • Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.
Study Product, Dose, Route, Regimen and duration of administration	Chemotherapy regimen: at the discretion of the treating physician, based also on patient's features and preferences. Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard", with activity documented preferentially through a published phase III clinical trial. Endocrine therapy: ▪ non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI, or who relapsed more than 24 months from the end of adjuvant AI ▪ non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed less than 24 months from the end of adjuvant steroidal AI ▪ steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed less than 24 months from the end of adjuvant non-steroidal AI
Reference therapy	The standard reference therapy is chemotherapy, administered until the achievement of maximum response or until interruption for toxicity or patient refusal or disease progression, followed by an AI.
Statistical Methodology	Assuming a median PFS of 6 months for patients undergoing first-line treatment for locally advanced or metastatic breast cancer, in order to detect an increase of PFS of 3 months (from 6 to 9 months, hazard ratio 0.67) in patients receiving concomitant chemo-endocrine therapy, with a two-tail logrank test at 90% power and 5% significance level, 150 patients must be enrolled in each arm (300 patients overall, with 256 expected events) in a period of 24 months, with further 12 months of follow up. Comparisons between the two treatment arms will be performed using the stratified log rank test and building Cox proportional-hazard models, with or without inclusion of the main know prognostic and predictive factors.

Amended text

Title	**Group sequential response adaptive randomized clinical trial of concomitant chemotherapy plus endocrine therapy versus cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy for advanced hormone receptor-positive, HER2-negative breast cancer**~~Randomized clinical trial of concomitant chemoendocrine therapy versus chemotherapy followed by endocrine therapy as first line treatment of luminal B metastatic breast cancer.~~
Short Title/ Acronym	**CDK4/6-inhibitor or chemotherapy, in combination with ENDOcrine therapy, for advanced breast cancer / KENDO** ~~CHemo-ENDOcrine therapy in advanced breast cancer / CHENDO~~
Protocol Code	IRST174.19 Identifier Code: L2P1388
Phase	Phase ~~3~~**2**

Study Design	Prospective, open label, multicenter, **group sequential response adaptive** randomized phase ~~III~~**2** study, comparing two ~~strategies as first chemotherapy-based~~treatment**s** for locally advanced or metastatic luminal breast cancer: - Arm A: concomitant **cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (aromatase inhibitor [AI] or fulvestrant)**~~chemotherapy plus an aromatase inhibitor (AI); the AI must continue until disease progression or toxicity or patient refusal; chemotherapy may be stopped after achievement of maximum response (generally after at least about 3-6 months of treatment) or in case of toxicity or patient refusal.~~ - Arm B: chemotherapy **plus endocrine therapy (AI or fulvestrant, administered either concomitantly from the beginning of chemotherapy or sequentially after 4-6 months of chemotherapy)** ~~followed by an AI at the time of progression to chemotherapy (if an endocrine therapy is deemed indicated by the treating physician) or as maintenance therapy after achieving maximum response to chemotherapy (generally after at least about 3-6 months of treatment) or after stopping chemotherapy for toxicity or patient refusal.~~**Treatments will continue until disease progression or toxicity or patient refusal.** **Cross-over to the other treatment arm is encouraged (although not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy.**
Back ground and Rationale for study	**Although metastatic luminal breast cancer is most often treated with endocrine agents as first-line therapy, chemotherapy may be useful in specific conditions, and is eventually administered to most patients after the onset of endocrine resistance. The choice and sequence of treatments for metastatic luminal breast cancer depends on responsiveness to previous (e.g. adjuvant) therapies and on biological and clinical features. Chemotherapy is used in earlier lines of treatment in presence of signs of disease aggressiveness, such as short disease-free interval, elevated Ki67 (preferably, if available, on a metastatic biopsy), low expression of hormone receptors (HRs), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms.** **Several new targeted agents are being developed in breast cancer, and some of them have been shown to improve the efficacy of endocrine therapy when given in combination with endocrine agents, and to overcome or delay the development of endocrine-resistance. The cyclin-dependent Kinase 4 and 6 (CDK4/6) inhibitors (palbociclib, ribociclib, abemaciclib) significantly improve progression-free survival when given in combination with an aromatase inhibitor (AI) as first-line treatment of endocrine-sensitive metastatic breast cancer, or when given in combination with fulvestrant in patients with HR-positive metastatic breast cancer after progression to an aromatase inhibitor. Therefore, a combination of an endocrine agent (AI or fulvestrant) and a CDK4/6 inhibitor has become the preferred first-line treatment in patients with HR-positive metastatic breast cancer, except in cases of very indolent disease or poor performance status, when endocrine therapy alone is still preferred, or in cases of very aggressive disease which may still be treated with first-line chemotherapy. The combinations of CDK4/6 inhibitors and endocrine therapy could potentially replace chemotherapy alone in some cases of aggressive disease, and studies comparing the two strategies are ongoing.** **Preclinical studies show a synergism between chemotherapy and some endocrine**

agents such as AIs and fulvestrant. Therefore, a combination of chemotherapy and endocrine therapy could be a further, potentially very active, treatment strategy in metastatic luminal breast cancer.

The choice between a chemotherapy-based and a CDK4/6 inhibitor-based treatment remains particularly controversial in patients with doubtful endocrine sensitivity, e.g. due to a low expression of estrogen receptors (ERs), or of primary endocrine resistance, indicated by a short disease-free interval.

We plan to conduct a phase II group sequential response adaptive randomized clinical trial comparing the combination of chemotherapy plus endocrine therapy with CDK4/6 inhibitors plus endocrine therapy in patients with advanced HR-positive, HER2-negative breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option.

The main aim is to assess if a combination of chemotherapy and endocrine therapy is superior to CDK4/6 inhibitors plus endocrine therapy in terms of progression-free survival. Secondary aims are comparing the two treatment arms in terms of disease control rate (objective response or stable disease after 3 months of treatment), objective response rate, overall survival, toxicity and patient reported outcomes. In a biological correlative study, the expression and mutational/ Copy Number Variation (CNV) profiles of genes involved in pathways subtending cell cycle progression (cyclin D – CDK4/6 – Rb), as targets of CDK4/6 inhibitors, and response to chemotherapy (p53, p38, DNA damage response) will be assessed on tumor specimens. The same gene mutational profiles and CNVs will be assessed on Circulating Tumor Cells (CTC) with analysis at single cell level. Also, on a subgroup of patients enrolled at Meldola, Forlì and Cesena, the expression profiles of genes involved in the pathways of interest (cyclin D – CDK4/6 – Rb, p53, p38, DNA damage response) will be assessed on CTCs at single cell level. ~~Although metastatic luminal breast cancer is most often treated with endocrine therapy as initial treatment, in some cases chemotherapy may be required, either at first metastatic relapse, or after failure of endocrine therapy.~~

~~This applies particularly to the luminal B breast cancer subtype, characterized by expression of estrogen receptors but high proliferative index and/or low expression of progesterone receptors, whose prognosis is worse, and responsiveness to endocrine therapy poorer, compared with the luminal A counterpart.~~

~~In some clinical conditions also luminal A breast cancer, characterized by expression of estrogen and progesterone receptors with low proliferative index, might require chemotherapy.~~

~~In these cases, it remains unknown if the concomitant administration of an endocrine agent to chemotherapy might improve results compared to chemotherapy alone.~~

Timelines	Estimated duration for the ~~main~~**amended** protocol: ~~4 years~~ **39 months** Study start (FPFV): ~~05~~**12**/201~~8~~**7** Recruitment end (LPFV): ~~04~~**10**/~~2019~~**2020** Follow-up period end date (LPLV): ~~04~~**02**/~~2020~~**2022**
Study Centers	Multi-center study

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Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0

Objectives and outcome measures	Primary	**To compare the efficacy of concomitant CDK4/6 inhibitor plus endocrine therapy versus chemotherapy plus endocrine therapy (administered either concomitantly from the beginning or sequentially) in terms of progression-free survival (PFS).**~~To compare the efficacy of concomitant chemotherapy and AI versus chemotherapy followed by AI in terms of progression free survival (PFS).~~

	Secondary	To compare between treatment arms:
		• quality of life (EORTC QLQ-C30 and QLQ-BR23)
		• toxicity (CTCAE version ~~4~~5.0~~3~~)
		• time to treatment failure
		• ~~time to progression to the therapeutic strategy~~
		• best response rate
		• duration of response
		• clinical benefit rate
		• overall survival (OS)
		• **PFS and clinical benefit with the subsequent line of treatment after cross-over: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy**
		• correlative biomarkers of response **to CDK4/6 inhibitors and** chemotherapy~~and endocrine therapy~~:
		   – tissue markers (on the primary tumor and / or metastatic tissue)
		   – circulating markers (e.g. CTCs, ctDNA)
		To compare, within the experimental arm, PFS with concomitant versus sequential administration of chemotherapy and endocrine therapy (exploratory endpoint)

Number of Subjects		**Actual sample size will depend on the possibility of early stopping for efficacy at any of the two planned interim analyses. However, at the most an overall sample size of 150 patients is planned.** Patients will be allocated according to group sequential response adaptive randomization.~~300 patients overall (150 patients in each treatment arm)~~
Diagnosis and Main Eligibility Criteria		Patient population: postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not) with **HR-positive,** HER2-negative, locally advanced or metastatic ~~luminal~~ breast cancer, ~~candidate to their first chemotherapy-based treatment for advanced disease~~**with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.** Inclusion criteria: • ~~Age ≥ 18 years.~~ • Histological diagnosis of ~~HER2-negative luminal~~**HR-positive (ER ≥10% of tumor cells), HER2-negative (according to ASCO guidelines 2018)** breast cancer ~~(ER ≥10% of tumor cells)~~, determined by local laboratory on most recent available tumor tissue. • Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as "advanced breast cancer (ABC)"). • **At least one of the following signs of disease aggressiveness:** o ~~Candidate to chemotherapy-based treatment per the investigator best~~

judgment; e.g. because of disease aggressiveness, short disease-free interval, elevated Ki67 [if available on a metastatic site], low expression of hormone receptors, extended visceral involvement, visceral involvement at risk for organ failure, uncontrolled symptoms), according to Associazione Italiana di Oncologia Medica (AIOM) Guidelines (2016 edition). **the main criteria are a low expression of ER (10% ≤ ER < 50%) and/or a relapse while on the first 2 years of adjuvant endocrine therapy or disease progression (PD) within the first 6 months of first-line endocrine therapy for ABC**

- o **Other tumor characteristics of aggressiveness that make the patient potentially candidate to chemotherapy, according to the guidelines of the Italian Association of Medical Oncology [AIOM guidelines 2017], such as: elevated Ki67 (preferably documented, if available, on a metastatic biopsy), low expression of hormone receptors (e.g. progesterone receptor <20%), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms; these patients are eligible if chemotherapy is considered a suitable option by the treating physician.**

- Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not).
- Measurable disease according to RECIST 1.1 criteria, or not measurable but evaluable disease.
- **Any prior adjuvant chemotherapy or endocrine therapy**
- No prior chemotherapy for advanced disease.
- Up to ~~two~~ **one** prior line of endocrine therapy for ABC~~, as well as targeted therapies (such as palbociclib and/or everolimus or investigative targeted therapies) administered as part of a prior hormonal regimen for ABC, are allowed.~~
- **Age ≥ 18 years.**
- Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).
- Adequate organ (renal, hepatic, bone marrow, cardiac) functions.
- Female participants of child bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.
- Participant is willing and able to give informed consent for participation in the study.

Exclusion criteria:
- Any prior chemotherapy **or CDK4/6 inhibitor** for advanced breast cancer
- **More than 1 prior line of endocrine therapy for ABC.** ~~Resistance to both non-steroidal and steroidal aromatase inhibitors, eg patients who progressed while on or within 12 months after the end of an aromatase inhibitor in the adjuvant setting and who progressed while on an aromatase inhibitor (of a different class) in the metastatic setting, or patients who progressed to both classes of aromatase inhibitors administered as two distinct lines of therapy for metastatic disease.~~
- Patients who have not recovered from adverse events due to prior therapies to

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grade ≤1 (excluding alopecia).
- Active central nervous system metastases.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to the ~~chemotherapeutic or endocrine agent~~**drug**s used in the study.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection~~(patients with history of hepatitis B must undergo prophylactic therapy with lamivudine or other agent according to infectious disease consultation)~~, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.

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Study Product, Dose, Route, Regimen and duration of administration	Chemotherapy regimen: at the discretion of the treating physician **(treatment of physician choice, TPC)**, based also on patient's features and preferences. Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard"~~, with activity documented preferentially through a published phase III clinical trial~~. **Chemotherapy regimens will be classified as:** ▪ **anthracycline + taxane,** ▪ **taxane,** ▪ **anthracycline,** ▪ **capecitabine / fluoropyrimidines,** ▪ **others.** Endocrine therapy: ▪ non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI~~, or who relapsed more than 24 months from the end of adjuvant AI~~ ▪ non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed ~~less than 24~~ **while on** ~~months from the end of~~ adjuvant steroidal AI ▪ steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed ~~less than 24~~ **while on** ~~months from the end of~~ adjuvant non-steroidal AI ▪ **fulvestrant in women not pretreated with fulvestrant for advanced disease** **CDK4/6 inhibitor:** ▪ **palbociclib** ▪ **ribociclib** ▪ **abemaciclib** **Any cytotoxic or endocrine agent to which a patient was exposed in the adjuvant setting may be administered within this study only if PD occurred at least 12 months after the end of that drug.**
Reference therapy	The standard reference therapy is ~~chemotherapy, administered until the achievement of maximum response or until interruption for toxicity or patient refusal or disease progression, followed by an AI~~**CDK4/6 inhibitor plus endocrine therapy**.

Statistical Methodology	**The patients will be allocated according to block randomization until two events are observed in each arm, and then according to the time-to-event adaptation (Zhang and Rosenberger, 2007) of the group sequential Doubly-adaptive Biased Coin Design (DBCD) whose allocation probabilities are computed at the end of the block randomization and after around 70% and 85% of the 150 maximum patients are enrolled during a 23 month period. At these last two (i.e. after 105 and 128 patients, respectively), interim analysis on efficacy (Zhu and Hu, 2010) will be carried out allowing for early stopping. Assuming for the survival times an exponential distribution parametrized in terms of its expected value, the null hypothesis of equality of PFS versus a higher one for arm B ($H_0: \theta_A = \theta_B$; $H_1: \theta_B > \theta_A$) will be tested by means of the nonparametric log-rank test with a 10% significance level. The adoption of the Lan and DeMets (1983) α-spending function for determining the upper boundaries allows to preserve the nominal level throughout the two interim analyses. At the end of the 16-month follow up, administrative censoring is introduced. Therefore, the total study duration is 39 months.** **Simulations carried out assuming different scenarios showed good operating characteristics, especially when compared with the ones of the usual complete randomization (CR) coupled with the same test statistics on a fixed 150 patient sample size. Previous results on palbociclib and fulvestrant combination in second line (Paloma 3 Study, supplementary material of Turner NC, NEJM 2018) and the characteristics of our target population lead us to assume a median PFS of 8 and 12 months for arm A and B, respectively. Under this scenario, for a sample size of at the most 150 patients, the proposed design strategy has led to a simulated power of 0.911 compared with a 0.717 one for the CR design. Moreover, a reduced expected sample size (ESS) of 121.259 patients is observed due to early stopping for efficacy, whereas CR forces all 150 patients to be equally assigned to both arms. Furthermore, assuming a median PFS of 8 and 12 months for Arm A and B, respectively, simulations showed that group sequential DBCD allocates around 56% of the patients to Arm B.** ~~Assuming a median PFS of 6 months for patients undergoing first-line treatment for locally advanced or metastatic breast cancer, in order to detect an increase of PFS of 3 months (from 6 to 9 months, hazard ratio 0.67) in patients receiving concomitant chemo-endocrine therapy, with a two-tail logrank test at 90% power and 5% significance level, 150 patients must be enrolled in each arm (300 patients overall, with 256 expected events) in a period of 24 months, with further 12 months of follow up.~~ ~~Comparisons between the two treatment arms will be performed using the stratified log rank test and building Cox proportional-hazard models, with or without inclusion of the main know prognostic and predictive factors.~~

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5) STUDY SCHEMA, page 11

Original text

Trial scheme

Disease:	**Stratify**	**Treatment**
Luminal breast cancer at first chemotherapy-based treatment for ABC	▪ type of CT ▪ type of AI ▪ previous ET for ABC	

Arm A: CT + AI (CT until max benefit or toxicity or patient refusal or PD; AI until PD or toxicity or patient refusal)

Patient exit study at first PD

⟶   **Randomization**

Treatments:
CT:
- anthracyclines + taxanes
- taxanes
- anthracyclines
- fluoropyrimidines
- others

ET:
- non-steroidal AI
- steroidal AI

Arm B: CT (until max benefit or toxicity or patient refusal or PD) → AI (until PD or toxicity or patient refusal)

Patient exit study: after first PD if, per clinical judgment, the patient is not candidate to receive AI; after second PD if the patient received AI

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Amended text

Trial scheme

Disease:
HR-positive, HER2-negative locally advanced or metastatic **breast cancer (ABC)**, with features of aggressiveness

Treatment:

Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy

Permuted Block Randomization
(until two relapses are experienced in both arms)

Response Adaptive Randomization (the allocation probabilities updating process will take place after 105 and 128 patients are enrolled along with interim analysis on efficacy allowing for early stopping of the trial)

Treatments:
CT:
- anthracyclines + taxanes
- taxanes
- anthracyclines
- fluoropyrimidines
- others

ET:
- non-steroidal AI
- steroidal AI
- fulvestrant

CKK4/6 inhibitor:
- palbociclib
- ribociclib
- abemaciclib

Arm B: chemotherapy plus endocrine therapy

Treatments will continue until disease progression or toxicity or patient refusal

Cross-over is encouraged (although not mandatory)

	Protocol Code: IRST174.19
SERVIZIO SANITARIO REGIONALE	Identifier Code: L2P1388
EMILIA-ROMAGNA	Date and Version: 09/11/2018 – Amendment 1.0
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori	
Istituto di Ricovero e Cura a Carattere Scientifico	

6) ABBREVIATIONS, page 12

Original text

...

DFS   Disease Free Survival
ECG   Electrocardiogram

...

Amended text

...

DFS   Disease Free Survival
DFI   **Disease Free Interval**
ECG   Electrocardiogram

...

7) Section 1.1 Background, page 17

Original text

…
For practical purposes, intrinsic breast cancer subtypes are approximated using clinicopathological criteria [Goldhirsch A 2011, Goldhirsch A 2013], and luminal B tumors are often identified as ER-positive with high Ki67 [Cheang MC 2009], or ER-positive and HER2-positive [Goldhirsch A 2011, Goldhirsch A 2013]. Other studies have identified a low expression of progesterone receptors (PgR) as criteria to separate luminal B from luminal A tumors [Prat A 2013]. In this study, we will consider only HER2-negative luminal B tumors, defined as ER-positive (>10% of tumor cells) and at least one of the following: PgR-negative (≤20%) or Ki67≥ 20%.
Prognosis of luminal B breast cancer is significantly worse compared to luminal A tumors and overall survival (OS) of untreated cases is more similar to that of basal-like and HER2-enriched subtypes [Hu Z 2006; Ades F 2014]. Luminal B tumors are less responsive to chemotherapy compared with HER2-enriched and basal-like subtypes, but more responsive than luminal A tumors, as highlighted by studies of neoadjuvant chemotherapy, showing different rates of pathological complete remissions [Esserman LJ 2012].
… In the adjuvant setting, benefit from chemotherapy added to endocrine therapy appears confined to patients with tumors with high recurrence score, a measure of recurrence risk based largely on levels of transcription of genes involved in cell proliferation and of ER-related genes [Albain KS 2010].
Luminal B tumors are characterized …
The profiles of gene mutations differ sharply between luminal A and B tumors, as shown by several works and foremost by The Cancer Genome Atlas Research Network [Cancer Genome Atlas Network 2012].
…

Amended text

…
For practical purposes, intrinsic breast cancer subtypes are approximated using clinicopathological criteria [Goldhirsch A 2011, Goldhirsch A 2013], and luminal B tumors are often identified as ER-positive with high Ki67 [Cheang MC 2009], or ER-positive and HER2-positive [Goldhirsch A 2011, Goldhirsch A 2013],. Other studies have identified **or ER-positive with** a low expression of progesterone receptors (PgR) as criteria to separate luminal B from luminal A tumors [Prat A

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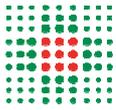
2013]. In this study, we will consider only HER2-negative luminal B tumors, defined as ER-positive (>10% of tumor cells) and at least one of the following: PgR ~~negative~~ **low** (≤20%) or Ki67 **high** (≥ 20%**)**.

Prognosis of luminal B breast cancer is significantly worse compared to luminal A tumors and ~~overall survival (OS) of~~ **in** untreated cases is more similar to that of basal-like and HER2-enriched subtypes [Hu Z 2006; Ades F 2014].

Treatments commonly used for luminal tumors include endocrine therapy, often combined with other targeted agents such as cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors, and chemotherapy. Responsiveness to these treatments varies between luminal A and luminal B tumors. Luminal B tumors are less responsive to chemotherapy compared with HER2-enriched and basal-like subtypes, but more responsive than luminal A tumors, as highlighted by studies of neoadjuvant chemotherapy~~, showing different rates of pathological complete remissions~~ [Esserman LJ 2012].

… In the adjuvant setting, benefit from chemotherapy added to endocrine therapy appears confined to patients with tumors with high recurrence score, a measure of recurrence risk based largely on levels of transcription of genes involved in cell proliferation and of ER-related genes [Albain KS 2010]. **CDK4/6 inhibitors work preferentially in luminal (including HER2-positive luminal) compared to other breast cancers subtypes. Most studies show comparable efficacy in luminal A and B tumors, but there are hints of increased benefit in tumors with luminal B features [Goetz MP 2018].**

Luminal B tumors are characterized …

The profiles of gene mutations differ sharply between luminal A and B tumors~~, as shown by several works and foremost by The Cancer Genome Atlas Research Network~~ [Cancer Genome Atlas Network 2012].

…

8) Section 1.2 Investigational Agents, page 19

Original text

Although metastatic luminal breast cancer is most often treated with endocrine therapy as initial treatment [Cardoso F 2014], in some cases chemotherapy may be required, either at first metastatic relapse, or after failure of one line of endocrine therapy. This applies particularly to the luminal B breast cancer subtype, whose prognosis is worse, and responsiveness to endocrine therapy poorer, compared with the luminal A counterpart. In some clinical conditions also luminal A breast cancer might require chemotherapy.

In these cases, it remains unknown if the concomitant administration of an endocrine agent to chemotherapy might improve results compared to chemotherapy alone.

Amended text

~~Although~~**Endocrine therapy is the preferred first-line treatment for** metastatic luminal breast cancer ~~is most often treated with endocrine therapy as initial treatment~~ [Cardoso F ~~2014~~**2018**], **and since the advent of CDK4/6 inhibitors a combination of an endocrine agent with a CDK4/6 inhibitor is commonly used as first-line (usually in combination with an AI, in patients naïve to AIs or relapsed >12 months after the end of adjuvant AIs, or with fulvestrant in patients relapsed while on or ≤12 months after the end of adjuvant AI) or second-line treatment (in combination with fulvestrant). According to the ESO-ESMO guidelines [Cardoso F 2018] the CDK4/6 inhibitors have a higher Magnitude of Clinical Benefit Scale (MCBS) score when used in the second line in combination with fulvestrant, where there is stronger evidence of health-related quality of life benefit, than when used in first-line.** ~~i~~In some cases **of advanced luminal breast cancer** chemotherapy may be required, either at first metastatic relapse, or after failure of one line of endocrine therapy. This applies particularly to the luminal B breast cancer subtype, whose prognosis is worse, and responsiveness to endocrine therapy poorer, compared with the luminal A counterpart. In some clinical conditions also luminal A breast cancer might require chemotherapy. **According to the Italian Association of Medical Oncology (AIOM) chemotherapy can be considered in the earlier lines of treatment in presence of signs of disease aggressiveness, such as short disease-free interval, elevated Ki67 (preferably, if available, on a metastatic biopsy), low expression of hormone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms [AIOM breast cancer guidelines]. Nonetheless, it is**

unknown if treatment with CDK4/6 inhibitors plus endocrine therapy could replace chemotherapy in these circumstances in more aggressive luminal tumors, and some clinical trials are ongoing to compare the two treatments. Preclinical studies show a synergism between chemotherapy and some endocrine agents such as AIs and fulvestrant. Therefore, a combination of chemotherapy and endocrine therapy could be a further, potentially very active, treatment strategy in advanced luminal breast cancer.

The choice between a chemotherapy-based and a CDK4/6 inhibitor-based treatment remains particularly controversial in patients with doubtful endocrine sensitivity, e.g. due to a low expression of estrogen receptors (ERs), or of primary endocrine resistance, indicated by a short disease-free interval.

We plan to conduct a phase II group sequential response adaptive randomized clinical trial comparing a combination of chemotherapy plus endocrine therapy with a combination of CDK4/6 inhibitors plus endocrine therapy in patients with advanced HR-positive, HER2-negative breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option.

~~In these cases, it remains unknown if the concomitant administration of an endocrine agent to chemotherapy might improve results compared to chemotherapy alone.~~

9) Section 1.3 Preclinical Data, page 20

Original text

… Treatment with the short-acting selective estrogen receptor modulator arzoxifene between courses of chemotherapy in MCF-7 breast cancer xenografts has been shown to inhibit tumor repopulation, improving the treatment effectiveness [Wu L 2005].

Amended text

… Treatment with the short-acting selective estrogen receptor modulator arzoxifene between courses of chemotherapy in MCF-7 breast cancer xenografts has been shown to inhibit tumor repopulation, improving the treatment effectiveness [Wu L 2005].

CDK4/6-inhibitors – preclinical data
Three CDK4/6 inhibitors have been approved so far by the FDA and EMA for treatment of patients with breast cancer: palbociclib, ribociclib and abemaciclib. CDK4/6 inhibitors bind the ATP pockets of CDK 4 and 6 with high selectivity (abemaciclib has some selectivity also for CDK9) and inhibit Cyclin D – CDK4/6 dependent phosphorylation of the Rb protein. Rb is active when unphosphorylated and inhibits the E2F transcription factor that is responsible for the transcription of genes that execute the cell cycle. Phosphorylation of Rb leads to its inactivation, releasing its inhibitory effect on the cell cycle and favoring its progression from G1 into the S phase. CDK4/6 inhibitors therefore prevent inactivation of Rb and block cell cycle progression, producing Rb de-phosphorylation at specific serine residues as their pharmacodynamic hallmark [Sherr CJ 2016].
Among human breast cancer cell lines representative of the different breast cancer subtypes, the ER-positive, luminal ones are the most sensitive, along with some HER2-amplified cell lines with luminal features, whereas cell lines with basal features are the most resistant [Finn RS 2009]. Both endocrine-sensitive and endocrine-resistant lines may respond to CDK4/6 inhibitors [Petrossian K 2016]. High levels of cyclin D1 and of Rb, and low levels of p16 were predictors of sensitivity to palbociclib in vitro [Finn RS 2009] and in ex vivo studies on primary human tumor cultures [Dean JL 2012b].

Endocrine resistance often involves activation of the Cyclin D – CDK4/6 – Rb pathway and alterations of this pathway are common in luminal breast cancer. A combination of endocrine therapy and CDK4/6 inhibitors is therefore a relevant therapeutic option in these tumors. Preclinical studies show that the combination of palbociclib and tamoxifen is synergic in ER-positive breast cancer cell lines and palbociclib monotherapy is active in tamoxifen-resistant MCF7 cell lines [Finn RS 2009]. CDK4/6 inhibitors have also shown synergism with fulvestrant [Alves CL 2016, O'Brien 2014] and AI [Petrossian K 2016, O'Brien 2014].

10) Section 1.4 Clinical Data to Date, page 23

Original text

Studies of chemo-endocrine therapy with tamoxifen

Several studies comparing chemo-endocrine therapy with chemotherapy alone in first line metastatic breast cancer have been conducted in the past, with controversial results (reviewed in [Pritchard KI 2008]). The vast majority of these studies involved the use of tamoxifen as endocrine agent, and of old chemotherapy regimens. Although some improvements in response rate and time to progression were reported, no study found an increase in OS. Moreover, most of the studies did not perform the correct comparison between the two treatment strategies, namely response rates and TTP with concomitant treatment compared with the overall response rate and TTP offered by the sequential use of chemotherapy followed by endocrine therapy. A study reporting such comparison showed increased response rate and time to treatment failure with CMF plus tamoxifen compared with CMF alone, but an increased time to failure to treatment strategy with CMF alone, followed, at the time of first progression, by CMF plus tamoxifen, and a nonsignificant improvement in OS with the last strategy.

Clinical studies comparing the concomitant versus sequential administration of chemotherapy and tamoxifen in the adjuvant setting reported conflicting results. The factorial SWOG-8814, INT0100 study randomized postmenopausal women with hormone-receptor-positive, node-positive breast cancer to six cycles of CAF (cyclophosphamide, doxorubicin, fluorouracil) chemotherapy plus 5 years of daily tamoxifen versus tamoxifen alone, and to CAF followed by tamoxifen (CAF-T) versus CAF plus concurrent tamoxifen (CAFT). The adjusted hazard ratio (HR) favoured CAF-T over CAFT, albeit without reaching statistical significance, for disease free survival (DFS) (HR 0.84, 0.70–1.01; p=0.061) and OS (HR 0.90, 0.73–1.10; p=0.30) [Albain KS 2009]. The GEICAM 9401 study randomized patients with node-positive breast cancer to receive tamoxifen concomitantly or sequentially to EC (epirubicin, cyclophosphamide) chemotherapy, reporting no differences in DFS, with adjusted HR (concomitant/sequential) 1.11 (95% CI 0.71–1.73; p=0.64) [Pico C 2004]. An Italian study randomized women with node-positive breast cancer to start tamoxifen during or after adjuvant chemotherapy consisting of alternating CEF (cyclophosphamide, epidoxorubicin, 5-fluorouracil) and CMF (cyclophosphamide, methotrexate, 5-fluorouracil) every 21 days for a total of 12 cycles, reporting no differences in OS, DFS and toxicity (sequential vs concurrent arm: HR of death 1.06, 95% CI 0.78-1.44, p=0.76; HR of relapse 1.16, 95% CI 0.88-1.52, p=0.36) [Bedognetti D 2011]. A further retrospective analysis of pre- and postmenopausal women who entered two Italian randomized trials (Gruppo Oncologico Nord-Ovest-Mammella Intergruppo studies) on adjuvant

chemotherapy and received either concomitant or sequential TAM showed no significant differences in the hazard of death (HR 1.13; 95% CI 0.78–1.64; p=0.534) and recurrence (HR 1.03; 95% CI 0.80–1.33; p=0.88) between the two groups, with a decreasing trend (p=0.015) in HR of death with increasing age, indicating that concomitant therapy might be more effective than sequential therapy in young patients. A meta-analysis from the Early Breast Cancer Trialists' Collaborative Group showed benefit from adding adjuvant tamoxifen to chemotherapy in estrogen receptor-positive breast cancer, both when tamoxifen was started concomitantly (recurrence rate ratio 0.62) and when started after chemotherapy (recurrence rate ratio 0.71), with no significan difference among these two strategies (indirect comparison) [EBCTCG 2011].

As a result of the lack of demonstration of superiority of the concurrent strategy, and based on the trend emerged in some studies, there is now consensus favoring the sequential administration of chemotherapy followed by tamoxifen in the adjuvant setting.

Studies of chemo-endocrine therapy with AIs

Only limited experience exists with concomitant chemotherapy and newer endocrine agents in clinical trials. A phase II study assessed the activity of a combination of chemotherapy with FEC (fluorouracil, doxorubicin and cyclophosphamide) for 6 cycles and concomitant exemestane 25 mg daily in postmenopausal patients with advanced breast cancer (ABC) [19]. On 23 patients there were 10 partial and 7 complete clinical responses (overall response rate 74%), a clinical benefit (objective response or stable disease) in 87% of the patients, and a median time to progression of 13.7 months. There was one case of pulmonary embolism. Other phase II studies have evaluated the combination of chemotherapy and endocrine agents in the neoadjuvant setting, both in premenopausal women receiving a GnRH analog [20] as endocrine agent or a GnRH analog plus letrozole [21], and in postmenopausal women receiving an AI (Torrisi, The Breast 2008)[22]. Overall, these studies yielded promising results, with objective clinical responses in about 75% of the patients and pathologic complete response rates ranging from 5 to 11%. The only randomized phase III trial reported in the literature compared a neoadjuvant chemotherapy with FEC (fluorouracil, doxorubicin and cyclophosphamide) for a median of 4 cycles with the same chemotherapy given concurrently with letrozole 2.5 mg daily [23]. On 101 patients randomized between the two arms, the pathologic complete response rate increased from 10% to 25% with the addition of letrozole, without worsening chemotherapy-related toxicity, although there were some added side effects typical of endocrine therapy, such as hot flushes.

Taken together, these evidences highlight the potential effectiveness of a combination of chemotherapy and endocrine therapy in patients with luminal B breast cancer. This provides the rational for our study, testing the hypothesis of superiority of concomitant chemotherapy and AI compared with chemotherapy followed by AI as first line treatment of metastatic luminal B breast cancer, aimed to improve the control of this disease.

Amended text

~~**Studies of chemo-endocrine therapy with tamoxifen**~~

~~Several studies comparing chemo-endocrine therapy with chemotherapy alone in first line metastatic breast cancer have been conducted in the past, with controversial results (reviewed in [Pritchard KI 2008]). The vast majority of these studies involved the use of tamoxifen as endocrine agent, and of old chemotherapy regimens. Although some improvements in response rate and time to progression were reported, no study found an increase in OS. Moreover, most of the studies did not perform the correct comparison between the two treatment strategies, namely response rates and TTP with concomitant treatment compared with the overall response rate and TTP offered by the sequential use of chemotherapy followed by endocrine therapy. A study reporting such comparison showed increased response rate and time to treatment failure with CMF plus tamoxifen compared with CMF alone, but an increased time to failure to treatment strategy with CMF alone, followed, at the time of first progression, by CMF plus tamoxifen, and a nonsignificant improvement in OS with the last strategy.~~

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As a result of the lack of demonstration of superiority of the concurrent strategy, and based on the trend emerged in some studies, there is now consensus favoring the sequential administration of chemotherapy followed by tamoxifen in the adjuvant setting.

Studies of chemo-endocrine therapy with AIs

Several studies comparing concomitant chemo-endocrine therapy with chemotherapy alone in first line metastatic breast cancer have been conducted in the past with the use of tamoxifen in combination with old chemotherapy regimens and have produced controversial results (reviewed in [Pritchard KI 2008]). Based on these findings and on studies of concomitant chemo-endocrine therapy with tamoxifen in the adjuvant setting [Pico C 2004, Albain KS 2009, Bedognetti D 2011, Del Mastro L Ann Oncol 2008; 19: 299-307, EBCTCG 2011], a sequential rather than concomitant strategy has become the standard of care.

Only limited experience exists with concomitant chemotherapy and newer endocrine agents in clinical trials. A phase II study assessed the activity of a combination of chemotherapy with FEC (fluorouracil, doxorubicin and cyclophosphamide) for 6 cycles and concomitant exemestane 25 mg daily in postmenopausal patients with advanced breast cancer (ABC) [**de la Haba-Rodriguez J 2010**19]. On 23 patients there were 10 partial and 7 complete clinical responses (overall response rate 74%), a clinical benefit (objective response or stable disease) in 87% of the patients, and a median time to progression of 13.7 months. There was one case of pulmonary embolism. Other phase II studies have evaluated the combination of chemotherapy and endocrine agents in the neoadjuvant setting, both in premenopausal women receiving a GnRH analog [**Torrisi R 2007**20] as endocrine agent or a GnRH analog plus letrozole [**Torrisi R 2008**21], and in postmenopausal women receiving an AI (Torrisi, The Breast 2008)[**Torrisi R 2008, Watanabe N 2010**22]. Overall, these studies yielded promising results, with objective clinical responses in about 75% of the patients and pathologic complete response rates ranging from 5 to 11%. The only randomized phase III trial reported in the literature compared a neoadjuvant chemotherapy with FEC (fluorouracil, doxorubicin and cyclophosphamide) for a median of 4 cycles with the same chemotherapy given concurrently with letrozole 2.5 mg daily [**Mohammadianpanah M 2012**23]. On 101 patients randomized between the two arms, the pathologic complete response rate increased from 10% to 25% with the addition of letrozole, without worsening chemotherapy-related toxicity, although there were some added side effects typical of endocrine therapy, such as hot flushes.

The combination of fulvestrant and metronomic capecitabine (1500-2000 mg daily continuously) was evaluated in a phase II study in 41 post-menopausal women with advanced breast cancer, reporting a median PFS of 14.98 months (95%CI 7.26-NR), median TTP of 26.94 months (95%CI 7.26-NR) and clinical benefit rate of 58.5%, with excellent tolerability [Schwartzberg LS 2014].

Taken together, these evidences highlight the potential effectiveness of a **the concomitant administration of** combination of chemotherapy and endocrine therapy**with the newer endocrine agents, such as AIs and fulvestrant,** in patients with **aggressive** luminal Bbreast cance**r**s.

Studies of CDK4/6 inhibitors in combination with endocrine therapy

All the CDK4/6 inhibitors that have undergone clinical development have been shown to improve results when combined with endocrine agents over endocrine therapy alone in patients with hormone receptor-positive, HER2-negative advanced breast cancer, both in first-line in combination with an AI (in patients naïve to AI or relapsed >12 months after the end of adjuvant AIs) or with fulvestrant (in patients relapsed while on or ≤12 months after the end of adjuvant AI) and in second-line (in combination with fulvestrant).

In the first-line setting, the phase III trial PALOMA 2 compared placebo + letrozole with palbociclib + letrozole in 666 post-menopausal patients, not previously treated for advanced breast cancer, showing an increase in median

PFS from 14.5 months in the placebo arm to 24.8 months in the palbociclib arm, with hazard ratio 0.58 (95% CI 0.46–0.72, p<0.000001) favoring palbociclib [Finn RS, 2016]. Similarly, the phase III study MONALEESA 2 compared placebo + letrozole with ribociclib + letrozole in 668 post-menopausal women with no prior systemic therapy for advanced disease, with a median duration of PFS not reached in the ribociclib group versus 14.7 months in the placebo group (hazard ratio 0.56; 95% CI, 0.43-0.72; p=0.000003) [Hortobagyi GN, 2016]. The phase III trial MONARCH 3 compared placebo + a non-steroidal AI (anastrozole or letrozole) with abemaciclib + a non-steroidal AI in 493 post-menopausal women with no prior systemic therapy for advanced disease, yielding a significant prolongation of PFS from a median of 14.7 months in the placebo arm to a median not reached in the abemaciclib arm (hazard ratio 0.54; 95% CI, 0.41-0.72; p=.000021) [Goetz MP, 2017].

In the second-line setting, the phase III study PALOMA-3 compared palbociclib plus fulvestrant versus placebo plus fulvestrant (with premenopausal women receiving also a GnRH agonist) in 521 women with HR-positive, HER2-negative advanced breast cancer progressing during or shortly after (≤12 months in the adjuvant and ≤1 month in the metastatic setting) prior endocrine therapy. Median PFS was 9.5 months with palbociclib versus 4.6 months with placebo, with hazard ratio 0.46 (95%CI 0.36-0.59, p<0.0001) [Turner NC, 2015]. The benefit in OS, a secondary endpoint, was not statistically significant (hazard ratio for death, 0.81; 95% CI, 0.64 to 1.03; P=0.09; absolute difference, 6.9 months) [Turner NC, 2018]. Similarly, the phase III study MONARCH 2 compared abemaciclib + fulvestrant versus placebo + fulvestrant (with premenopausal women receiving also a GnRH agonist) in 669 women who had progressed while receiving neo/adjuvant endocrine therapy, ≤12 months after the end of adjuvant endocrine therapy, or while receiving first-line endocrine therapy for advanced disease, showing a median PFS of 16.4 months with abemaciclib versus 9.3 months with placebo (hazard ratio 0.553; 95%CI 0.449-0.681; p=.001) [Sledge GW Jr, 2017].

The phase III trial MONALEESA-3 compared ribociclib + fulvestrant versus placebo + fulvestrant in 484 post-menopausal women with HR-positive, HER2-negative advanced breast cancer who were treatment naïve or had received up to one line of prior endocrine therapy in the advanced setting. Median PFS was 20.5 months with ribociclib versus 12.8 months with placebo (hazard ratio 0.593; 95% CI 0.480-0.732; p=.001) [Slamon DJ, 2018]. Similar results were observed in the subgroups of patients who were treatment naïve and in those pretreated.

The phase III MONALEESA-7 trial compared ribociclib + endocrine therapy (either tamoxifen or a non-steroidal aromatase inhibitor, all with goserelin) with placebo + endocrine therapy in 672 pre-menopausal women not previously treated with endocrine therapy for advanced disease. The median PFS was 23·8 months in the ribociclib group compared with 13·0 months in the placebo group (hazard ratio 0.55, 95%CI 0.44–0.69; p<0·0001) [Tripathy D, 2018].

All these trials show a clinically relevant improvement in PFS with the addition of a CDK4/6 inhibitor to endocrine therapy, which is generally accompanied by improvements in other endpoints such as the objective response rate. This highlights the potential of these regimens to substitute chemotherapy in cases of aggressive luminal tumors. While clinical trials comparing these two treatment modalities are ongoing, an indirect comparisons between CDK4/6 inhibitors and chemotherapeutic drugs done by means of a network meta-analysis shows improvements in PFS with CDK4/6 inhibitors compared to several chemotherapeutic agents [Wilson FR, 2017]. ~~This provides the rational for our study, testing the hypothesis of superiority of concomitant chemotherapy and AI compared with chemotherapy followed by AI as first line treatment of metastatic luminal B breast cancer, aimed to improve the control of this disease.~~

11)   Section 1.6 Rationale and Risk/Benefits, page 26

Original text

In some patients with metastatic luminal breast cancer chemotherapy may be required, either at first metastatic relapse, or after failure of endocrine therapy. In these cases, it remains unknown if the concomitant administration of an endocrine agent to chemotherapy might improve results compared to chemotherapy alone. Given the low toxicity profile of AI, it could be worthy to use them concomitantly with chemotherapy if the combination proves to be superior to chemotherapy alone.
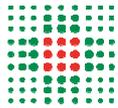
Amended text

~~In~~ **The best treatment approach for** ~~some~~ patients with ~~metastatic~~ **aggressive** luminal breast cancer**is still controversial and studies comparing endocrine therapy plus CDK4/6 inhibitors with** chemotherapy ~~may be required~~**are ongoing.**~~,~~ **Preclinical evidences of synergism between chemotherapy and newer endocrine agents suggest that a combination of chemotherapy plus endocrine therapy could be a further effective treatment for theses patients. We therefore plan a randomized clinical trial to test the superiority of a combination of chemotherapy plus endocrine therapy (experimental arm) versus the combination of CDK4/6 inhibitors plus endocrine therapy (standard arm), in patients with advanced luminal breast cancer. The target population is represented first and foremost by patients with primary resistance to endocrine therapy (e.g. relapse within 2 years of adjuvant endocrine therapy or within 6 months of first line endocrine therapy for advanced disease) or doubtful endocrine sensitivity (e.g. ER expression in less than 50% of tumor cells); secondly, any patient with signs of disease aggressiveness, such as elevated Ki67 (preferably, if available, on a metastatic biopsy), low or absent expression of progesterone receptors, extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms, are eligible if endocrine therapy alone is deemed inadequate by the treating physician and chemotherapy is considered a suitable option. Patients in the experimental arm will receive chemotherapy, with a regimen at the discretion of the treating physician, in combination with an endocrine agent (either AI or fulvestrant) started (at the discretion of the treating physician) concomitantly with chemotherapy or sequentially after 4-6 months of chemotherapy and continued until disease progression or toxicity or patient refusal.**
In case of superiority of the combination of chemotherapy plus endocrine therapy, this should be considered the first choise treatment for aggressive luminal tumors. If superiority is not established, the less toxic treatment should be considered as first choise. The comparison between concomitant versus sequential administration of chemotherapy and endocrine therapy will be a secondary exploratory endpoint.
The trial allows patients entered in the chemotherapy plus endocrine therapy arm to receive CDK4/6 inhibitors in subsequent lines, recommending cross over, therefore does not deny to these patients the opportunity to receive CDK4/6 inihibitors.
Given the low toxicity profile of the new endocrine agents, no relevant increase in toxicity is expected in the chemotherapy arm, but thromboembolic events, potentially favored both by chemotherapy and endocrine therapy, will be specifically monitored.
~~either at first metastatic relapse, or after failure of endocrine therapy. In these cases, it remains unknown if the concomitant administration of an endocrine agent to chemotherapy might improve results compared to chemotherapy alone. Given the low toxicity profile of AI, it could be worthy to use them concomitantly with chemotherapy if the combination proves to be superior to chemotherapy alone.~~

12)   Section 2 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS, page 28

Original text

The study will test the hypothesis of superiority of concomitant chemotherapy and AI compared with chemotherapy followed by AI as first chemotherapy-based treatment for locally advanced and metastatic luminal breast cancer.

Amended text

The study will test the hypothesis of superiority of ~~concomitant~~ **a combination of** chemotherapy **plus endocrine therapy** ~~and~~ (AI **or fulvestrant**) compared with ~~chemotherapy followed~~ **cyclin-dependent kinase 4/6 inhibitor plus endocrine therapy** (AI **or fulvestrant**) ~~as first chemotherapy-based treatment for~~ **in patients with** locally advanced and metastatic **HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness.**
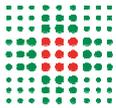
13)   Section 2.1 Primary Objective, page 28

Original text

To compare the efficacy of concomitant chemotherapy and AI versus chemotherapy followed by AI in terms of PFS.
The statistical hypothesis is that of superiority of the concomitant treatment (chemotherapy plus AI) compared with sequential treatment (chemotherapy followed by AI) in terms of PFS (see the section 11. Statistical Considerations).

Amended text

To compare the efficacy of ~~concomitant~~**a combination of** chemotherapy ~~and AI~~**plus endocrine therapy** versus ~~chemotherapy followed by AI~~**CDK4/6 inhibitors plus endocrine therapy** in terms of PFS.
The statistical hypothesis is that of superiority of the ~~concomitant~~ **combination of** chemotherapy plus ~~AI)~~**endocrine therapy** compared with ~~sequential treatment (chemotherapy followed by AI)~~**CDK4/6 inhibitor plus endocrine therapy** in terms of PFS (see the section 11. Statistical Considerations).

14)   Section 2.2 Secondary Objective, page 28

Original text

   To compare between the two treatment strategies:
- quality of life
- toxicity
- time to treatment failure (TTF)
- time to progression to the therapeutic strategy
- best response rates overall and during chemotherapy
- duration of response
- clinical benefit rate
- overall survival
- To assess correlative biomarkers of response to chemotherapy and endocrine therapy:
o tissue markers (on the primary tumor and / or metastatic tissue)
   circulating markers (e.g. CTCs, ctDNA)

Amended text

   To compare between the two treatment ~~strategies~~**arms**:
- quality of life **(EORTC QLQ-C30 and QLQ-BR23)**
- toxicity **(CTCAE version 4~~5.0~~**.0~~3~~**)**
- time to treatment failure (TTF)
- ~~time to progression to the therapeutic strategy~~
- best response rat~~es overall and during chemotherapy~~
- duration of response
- clinical benefit rate
- overall survival
- **PFS and clinical benefit with the subsequent line of treatment after cross-over: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy**
- To assess correlative biomarkers of response to chemotherapy and ~~endocrine therapy~~**CDK4/6 inhibitors**:

o   tissue markers (on the primary tumor and / or metastatic tissue)
o   circulating markers (e.g. CTCs, ctDNA)

To compare, within the experimental arm, PFS with concomitant versus sequential administration of chemotherapy and endocrine therapy (exploratory endpoint)

15)   Section 2.5 Secondary endpoints/outcomes, page 29

Original text

…

- TTF: the time from randomization to treatment discontinuation for any reason, including disease progression, treatment toxicity, patient refuse or death.
- time to progression to the therapeutic strategy, defined as follows:
    o   concurrent arm (arm A): time to first disease progression
    o   sequential arm (arm B):
    - time to first disease progression if, per clinical judgment, the patient is not candidate to receive AI;
    - time to second disease progression if the patient received AI
- best objective (partial or complete) response rate according to RECIST 1.1 (see Appendix D), defined as:
    o   percentage of responses to the combined treatment (arm A)
    o   overall percentage of response (during chemotherapy or during AI) with the sequential treatment (arm B)
- duration of response: time from documentation of tumor response to disease progression
- clinical benefit rate (CBR): the percentage of patients who achieved complete response, partial response or stable disease lasting longer than 24 weeks
- overall survival (OS): time from randomization until death for any cause

correlative biomarkers: assessed on baseline tumor specimens (from primary tumor or metastatic biopsies) and blood samples collected at baseline and at different timepoints until evidence of disease progression.

Amended text

…

- TTF: the time from randomization to treatment discontinuation for any reason, including disease progression, treatment toxicity, patient refus**ale** or death.
- time to progression to the therapeutic strategy, defined as follows:
    o   concurrent arm (arm A): time to first disease progression
    o   sequential arm (arm B):
    - time to first disease progression if, per clinical judgment, the patient is not candidate to receive AI;
    - time to second disease progression if the patient received AI
- best objective (partial or complete) response rate according to RECIST 1.1 (see Appendix D) , defined as:percentage of responses to the combined treatment (arm A)
- overall percentage of response (during chemotherapy or during AI) with the sequential treatment (arm B)
- duration of response: time from documentation of tumor response to disease progression
- clinical benefit rate (CBR): the percentage of patients who achieved complete response, partial response or stable disease lasting longer than 24 weeks
- overall survival (OS): time from randomization until death for any cause
- **PFS and clinical benefit with the subsequent line of treatment after cross-over: as above, calculated from the date of start of the subsequent treatment line**
- correlative biomarkers: assessed on baseline tumor specimens (from primary tumor or metastatic biopsies) and blood samples collected at baseline and at different timepoints until evidence of disease progression.

16) Section 3.1 Summary of Trial Design, page 30

Original text

This is a prospective, open label, multicenter, randomized phase III study, comparing two strategies as first chemotherapy-based treatment for locally advanced or metastatic luminal breast cancer:

- Arm A: concomitant chemotherapy plus an AI; the AI must continue until disease progression or toxicity or patient refusal; chemotherapy may be stopped after achievement of maximum response (generally after at least about 3-6 months of treatment) or in case of toxicity or patient refusal.

- Arm B: chemotherapy followed by an AI at the time of progression to chemotherapy (if an endocrine therapy is deemed indicated by the treating physician) or as maintenance therapy after achieving maximum response to chemotherapy (generally after at least about 3-6 months of treatment) or after stopping chemotherapy for toxicity or patient refusal.

Patients will be enrolled and randomized after the screening period (which should last no longer than 28 days), will be followed during treatment with chemotherapy (with visits for evaluation of toxicity at each cycle) and during treatment with AI (with visits at least every 3 months for disease and toxicity evaluation) until disease progression (including the second disease progression for patients in arm B who receive the AI after progression to chemotherapy) or until exit from the study for toxicity or patient refusal.

Patients' enrolment is expected to occur over a period of 24 months, with further 12 months of follow up.

Trial scheme

Disease:
Luminal breast cancer at first chemotherapy-based treatment for ABC

Stratify
type of CT
type of AI
previous ET for ABC

Treatment
Arm A: CT + AI (CT until max benefit or toxicity or patient refusal or PD; AI until PD or toxicity or patient refusal)

Patient exit study at first PD

Randomization

Treatments:
CT:
anthracyclines + taxanes
taxanes
anthracyclines
fluoropyrimidines
others

ET:
non-steroidal AI
steroidal AI

Arm B: CT (until max benefit or toxicity or patient refusal or PD) → AI (until PD or toxicity or patient refusal)

Patient exit study: after first PD if, per clinical judgment, the patient is not candidate to receive AI; after second PD if the patient received AI

Amended text

This is a prospective, open label, multicenter, **phase 2, group sequential response adaptive** randomized ~~phase III study~~**trial**, comparing two ~~strategies as first chemotherapy based~~**combination** treatment**s** for locally advanced or
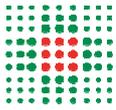
metastatic ~~luminal~~ **HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness:**

  - Arm A: concomitant **CDK4/6 inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant)**~~chemotherapy plus an AI; the AI must continue until disease progression or toxicity or patient refusal; chemotherapy may be stopped after achievement of maximum response (generally after at least about 3-6 months of treatment) or in case of toxicity or patient refusal.~~

  - Arm B: chemotherapy ~~followed by an~~ **plus endocrine therapy (AI or fulvestrant). The chemotherapy regimen will be at the discretion of the treating physician and will be administered for at least 4-6 months (unless there is toxicity or disease progression). The endocrine agent can be started concomitantly with chemotherapy or sequentially, after stopping chemotherapy.**

Treatments will continue until disease progression or toxicity or patient refusal.

Cross-over to the other treatment arm is suggested (but not mandatory) after disease progression: CDK4/6 inhibitors plus endocrine therapy in patients treated with chemotherapy plus endocrine therapy within the study, chemotherapy (with or without endocrine therapy) in patients treated with CDK4/6 inhibitors plus endocrine therapy within the study.

~~at the time of progression to chemotherapy (if an endocrine therapy is deemed indicated by the treating physician) or as maintenance therapy after achieving maximum response to chemotherapy (generally after at least about 3-6 months of treatment) or after stopping chemotherapy for toxicity or patient refusal.~~

Patients will be enrolled and randomized after the screening period (which should last no longer than 28 days), will be followed during treatment with ~~chemotherapy (with~~ visits for evaluation of toxicity at each cycle and ~~during treatment with AI (with visits~~ **with imaging for disease evaluation** at least every 3 months ~~for disease and toxicity evaluation)~~ until disease progression ~~(including the second disease progression for patients in arm B who receive the AI after progression to chemotherapy)~~ or until exit from the study for toxicity or patient refusal.

Patients' enrolment is expected to occur over a period of ~~24~~ **23** months, with further ~~12~~ **16** months of follow up.
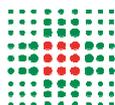
Trial scheme

Disease:

Luminal **(**HER2-negative**)** locally advanced or metastatic **breast cancer (ABC)**, with features of aggressiveness

Permuted Block Randomization (until two relapses are experienced in both arms)

Response Adaptive Randomization (the allocation probabilities updating process will take place after 105 and 128 patients are enrolled along with interim analysis on efficacy allowing for early stopping of the trial)

Treatments:
CT:
anthracyclines + taxanes
taxanes
anthracyclines
fluoropyrimidines
others
ET:
non-steroidal AI
steroidal AI
fulvestrant
CKK4/6 inhibitor:
palbociclib
ribociclib
abemaciclib

Treatment

Arm A: concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor plus endocrine therapy

Arm B: concomitant chemotherapy plus endocrine therapy

Treatments will continue until disease progression or toxicity or patient refusal

Cross-over is encouraged (although not mandatory)

17)   Section 4 STUDY POPULATION, page 32

Original text

The study will enroll postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not), with HER2-negative, luminal breast cancer, candidate to their first chemotherapy-based treatment for advanced disease.
…

Amended text

The study will enroll postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not), **with HR-positive,** HER2-negative, ~~luminal~~ breast cancer, ~~candidate to their first chemotherapy-based treatment for advanced disease~~ **with doubtful endocrine sensitivity or primary endocrine resistance or other signs of disease aggressiveness**.
…

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EMILIA-ROMAGNA
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0
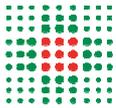
18) Section 4.1 Inclusion Criteria, page 32

Original text

1. Age ≥ 18 years.
2. Histological diagnosis of HER2-negative luminal breast cancer (ER >10% of tumor cells), determined by local laboratory on most recent available tumor tissue.
3. Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as advanced breast cancer, ABC).
4. Candidate to chemotherapy-based treatment per the investigator best judgment; e.g. because of disease aggressiveness, short disease-free interval, elevated Ki67 [if available on a metastatic site], low expression of hormone receptors, extended visceral involvement, visceral involvement at risk for organ failure, uncontrolled symptoms), according to Associazione Italiana di Oncologia Medica (AIOM) Guidelines (2016 edition).
5. Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not). Postmenopausal status is defined as:
    a. bilateral, surgical oophorectomy
    b. age ≥60 years
    c. age <60 years, with amenorrhea >12 months and follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol concentrations within postmenopausal range
    d. age <60 years and previous simple hysterectomy, with FSH, LH and estradiol levels within the post-menopausal range at two consecutive assessments two weeks apart.
6. Measurable disease according to RECIST 1.1 criteria or non measurable but evaluable lesions.
7. No prior chemotherapy for ABC. Up to two prior lines of endocrine therapy for ABC, as well as targeted therapies (such as palbociclib and/or everolimus or investigative targeted therapies) administered as part of a prior hormonal regimen for ABC, are allowed.
8. Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).
9. Adequate organ (renal, hepatic, bone marrow, cardiac) functions.
10. Female participants of child-bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.
11. Participant is willing and able to give informed consent for participation in the study

Amended text

1. ~~Age ≥ 18 years.~~
1. Histological diagnosis of **HR-positive (ER ≥10% of tumor cells), HER2-negative (according to ASCO guidelines 2018)** ~~HER2-negative luminal~~ breast cancer ~~(ER >10% of tumor cells)~~, determined by local laboratory on most recent available tumor tissue.
2. Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as advanced breast cancer, ABC).
3. **At least one of the following signs of disease aggressiveness:**
    a. **the main criteria are a low expression of ER (10% ≤ ER < 50%) and/or or a relapse while on the first 2 years of adjuvant endocrine therapy or disease progression within the first 6 months of first-line endocrine therapy for ABC.**
    ~~Candidate to chemotherapy-based treatment per the investigator best judgment; e.g. because of disease aggressiveness, short disease-free interval, elevated Ki67 [if available on a metastatic site], low expression of hormone receptors, extended visceral involvement, visceral involvement at risk for~~
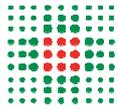
~~organ failure, uncontrolled symptoms), according to Associazione Italiana di Oncologia Medica (AIOM) Guidelines (2016 edition).~~

b. **Other tumor characteristics of aggressiveness that make the patient potentially candidate to chemotherapy, according to the guidelines of the Italian Association of Medical Oncology [AIOM guidelines 2017], such as: elevated Ki67 (preferably documented, if available, on a metastatic biopsy), low expression of hormone receptors (e.g. progesterone receptor <20%), extended visceral involvement or visceral involvement at risk for organ failure, uncontrolled symptoms; these patients are eligible if chemotherapy is considered a suitable option by the treating physician.**

4. Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not). Postmenopausal status is defined as:
    a. bilateral, surgical oophorectomy
    b. age ≥60 years
    c. age <60 years, with amenorrhea >12 months and follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol concentrations within postmenopausal range
    d. age <60 years and previous simple hysterectomy, with FSH, LH and estradiol levels within the post-menopausal range at two consecutive assessments two weeks apart.
5. Measurable disease according to RECIST 1.1 criteria or non-measurable but evaluable lesions.
6. **Any prior adjuvant chemotherapy or endocrine therapy**
7. No prior chemotherapy for ABC.
8. Up to ~~two~~ **one** prior line of endocrine therapy for ABC~~, as well as targeted therapies (such as everolimus or investigative targeted therapies) administered as part of a prior hormonal regimen for ABC, are allowed~~.
9. **Age ≥ 18 years**.
10. Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (see Appendix A).
11. Adequate organ (renal, hepatic, bone marrow, cardiac) functions.
12. Female participants of child-bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository.
13. Participant is willing and able to give informed consent for participation in the study

19)   Section 4.2 Exclusion Criteria, page 34

Original text

The participant may not enter the study if ANY of the following apply:
1. Any prior chemotherapy for ABC
2. Resistance to both non-steroidal and steroidal aromatase inhibitors, e.g. patients who progressed while on or within 12 months after the end of an aromatase inhibitor in the adjuvant setting and who progressed while on an aromatase inhibitor (of a different class) in the metastatic setting, or patients who progressed to both classes of aromatase inhibitors administered as two distinct lines of therapy for metastatic disease.
3. Patients who have not recovered from adverse events (AEs) due to prior therapies to grade ≤1 (excluding alopecia).
4. Active central nervous system metastases.
5. History of allergic reactions attributed to compounds of similar chemical or biologic composition to the chemotherapeutic or endocrine agents used in the study.
6. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (patients with history of hepatitis B must undergo prophylactic therapy with lamivudine or other agent according to infectious

disease consultation), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

7. Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.

Amended text

The participant may not enter the study if ANY of the following apply:

1. Any prior chemotherapy **or CDK4/6 inhibitor** for ABC.
2. **More than 1 prior line of endocrine therapy for ABC.**
3. ~~Resistance to both non-steroidal and steroidal aromatase inhibitors, e.g. patients who progressed while on or within 12 months after the end of an aromatase inhibitor in the adjuvant setting and who progressed while on an aromatase inhibitor (of a different class) in the metastatic setting, or patients who progressed to both classes of aromatase inhibitors administered as two distinct lines of therapy for metastatic disease.~~
4. Patients who have not recovered from adverse events (AEs) due to prior therapies to grade ≤1 (excluding alopecia).
5. Active central nervous system metastases.
6. History of allergic reactions attributed to compounds of similar chemical or biologic composition to the ~~chemotherapeutic or endocrine agents~~**drugs** used in the study.
7. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection ~~(patients with history of hepatitis B must undergo prophylactic therapy with lamivudine or other agent according to infectious disease consultation),~~ symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
8. Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years.

20)   Section 5.2 Screening and Eligibility Assessments, page 36

Original text

- Laboratory Tests:
  ....
- Serum Hepatitis assessment according to institutional practice (e.g. Hepatitis B surface antigen and Hepatitis C virus antibody).
- Serum or urine pregnancy test within one week prior to the start of study drug for women of child bearing potential.
  ...

Amended text

- Laboratory Tests:
  ....
- ~~Serum Hepatitis assessment according to institutional practice (e.g. Hepatitis B surface antigen and Hepatitis C virus antibody).~~
- Serum or urine pregnancy test within one week prior to the start of study drug for women of child bearing potential.
  ...
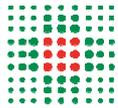
21) Section 5.4 Randomization, page 37

Original text

Patients will be randomized on a 1:1 allocation rate. Separated randomization lists, using a permuted block balanced procedure, will be generated for each participating center. The chemotherapy and endocrine regimens will be decided by the treating physician for each patient before randomization.
Treatment administration should begin within 72 hours of the date of randomization.
No blinding is planned.

Amended text

Patients will be randomized on a 1:1 allocation rate **according to block randomization (block of size two) until two events (i.e. disease progression or death) are experienced in both arms and then according to a group sequential DBCD [Hu F 2004] targeting Neyman allocation for time to event data [Zhang L 2007]. The allocation probabilities will be computed at the end of the block randomization and updated after 70% and 85% of the maximum 150 patients are enrolled (i.e. 105 and 128 patients). Central** ~~Ss~~eparated randomization lists~~, using a permuted block balanced procedure,~~ will be generated~~for each participating center~~ **at the start of the trial and the at each halt**. The chemotherapy**, CDK4/6 inhibitor** and endocrine ~~regimens~~ **agents** will be decided**chosen** by the treating physician for each patient before randomization.
Treatment administration should begin within 72 hours of the date of randomization.
No blinding is planned.

22) Section 5.6 Assessments during treatment period (cycle duration 21 or 28 days), page 38

Original text

<u>Before each chemotherapy cycle</u> (within 36 hours prior to treatment administration) the following evaluations must be performed:
- Interim medical history, including tumor-related signs and symptoms and new medical conditions
- Assessment of AEs (CTCAE version 4.03, Appendix C);
- Assessment of compliance with study drugs (oral chemotherapy and/or endocrine therapy)
- ...
- ECOG-PS, vital signs (including at least body temperature, resting blood pressure and cardiac frequency), weight.
- Laboratory Tests: CBC and serum chemistry

<u>Every 3 months</u> (every 4 cycles for 21-day chemotherapy regimens, every 3 cycles for 28-day chemotherapy regimens) the following evaluations must be performed:
- ...
- Blood samples for CTCs are to be collected 6-8 weeks after the beginning of study treatment, and 2-4 weeks after the last dose of chemotherapy.

Amended text

<u>Before each chemotherapy **or CDK4/6 inhibitor** cycle</u> (within 36 hours prior to treatment administration) the following evaluations must be performed:
- Interim medical history, including tumor-related signs and symptoms and new medical conditions

- Assessment of AEs (CTCAE version ~~4~~5.0~~3~~, Appendix **B**~~C~~);
- Assessment of compliance with study drugs (oral chemotherapy, **CDK4/6 inhibitor** and/or endocrine therapy)
- ...
- ECOG-PS, vital signs (including at least ~~body temperature,~~ resting blood pressure and cardiac frequency), weight.
- Laboratory Tests: CBC and serum chemistry

<u>Every 3 months</u> (every 4 cycles for 21-day chemotherapy regimens, every 3 cycles for 28-day chemotherapy **or CDK4/6 inhibitor** regimens) the following evaluations must be performed:
- ...
- Blood samples for CTCs are to be collected 6-8 weeks after the beginning of study treatment, and ~~2-4 weeks after the last dose of chemotherapy~~**at the end of treatment**.

23) Section 5.7 End of treatment assessments (within 30 days …), page 39

Original text

The following assessments will be completed within 30 days of the patient's last treatment administration:
- Interim medical history, including tumor-related signs and symptoms and new medical conditions
- Assessment of AEs (CTCAE version 4.03, Appendix B);
- Assessment of compliance with study drugs (oral chemotherapy and/or endocrine therapy)
- ...
- ECOG-PS, vital signs (including at least body temperature, resting blood pressure and cardiac frequency), weight.
- ...

Amended text

The following assessments will be completed within 30 days of the patient's last treatment administration:
- Interim medical history, including tumor-related signs and symptoms and new medical conditions
- Assessment of AEs (CTCAE version ~~4~~5.0~~3~~, Appendix B);
- Assessment of compliance with study drugs (oral chemotherapy**, CDK4/6 inhibitors** and/or endocrine therapy)
- ...
- ECOG-PS, vital signs (including at least ~~body temperature,~~ resting blood pressure and cardiac frequency), weight.
- ...

24) Section 5.8 Follow-up visits, page 41

Original text

Patients enrolled in arm A will be followed with tumor assessments until the first disease progression, in case of interruption of study treatment before progression due to toxicity or patient refusal.
Patients in arm B will be followed with tumor assessments until disease progression during chemotherapy and, for patients who receive an AI as subsequent treatment, until the second disease progression (during AI treatment).

After the EOT visit, all the patients will be followed every 3 months until disease progression and the following assessments will be performed at each Follow Up Visit:
- Interim medical history, including tumor-related signs and symptoms and new medical conditions
- Assessment of AEs (CTCAE version 4.03, Appendix B);
- Assessment of compliance with study drugs (oral chemotherapy and/or endocrine therapy)
- Assessment of concomitant medications (in particular if the patient started new medications, registering dosage, route of administration, start and end date and the clinical indication)
- Physical examination, with description (and measurement of the main diameter when feasible) or picture of superficial tumor lesions.
- ECOG-PS, vital signs (including at least body temperature, resting blood pressure and cardiac frequency), weight.
- Laboratory Tests: CBC and serum chemistry
- Tumor evaluations:
  o CT scan of chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted.
  o Other exams for tumor assessment if positive at baseline (e.g. CT or MRI of brain and involved bone segments in patients with known brain or bone metastasis) or as clinically indicated (suspected disease progression at new sites)

After progression to the therapeutic strategy (see 2.5 paragraph) , patients will be followed every 3 months according to clinical practice, and the subsequent lines of therapy and survival data will be registered. For these Survival Visits phone contacts are acceptable.
Every patient will be followed (follow up visits and survival visits) for 1 year after EoCT.

Amended text

~~Patients enrolled in arm A will be followed with tumor assessments until the first disease progression, in case of interruption of study treatment before progression due to toxicity or patient refusal.~~
~~Patients in arm B will be followed with tumor assessments until disease progression during chemotherapy and, for patients who receive an AI as subsequent treatment, until the second disease progression (during AI treatment).~~
After the EOT visit, all the patients will be followed **according to clinical practice. Data on response and TTP to the immediate subsequent line of treatment (particularly in case of cross-over) must be registered, along with survival data**~~every 3 months until disease progression and the following assessments will be performed at each Follow Up Visit:~~
- ~~Interim medical history, including tumor-related signs and symptoms and new medical conditions~~
- ~~Assessment of AEs (CTCAE version 4.03, Appendix B);~~
- ~~Assessment of compliance with study drugs (oral chemotherapy and/or endocrine therapy)~~
- ~~Assessment of concomitant medications (in particular if the patient started new medications, registering dosage, route of administration, start and end date and the clinical indication)~~
- ~~Physical examination, with description (and measurement of the main diameter when feasible) or picture of superficial tumor lesions.~~
- ~~ECOG-PS, vital signs (including at least body temperature, resting blood pressure and cardiac frequency), weight.~~
- ~~Laboratory Tests: CBC and serum chemistry~~
- ~~Tumor evaluations:~~
  o ~~CT scan of chest, abdomen and pelvis with contrast medium. In patients with proven allergy to iodinated contrast media, chest CT without contrast medium and MRI of the abdomen and pelvis (possibly wih contrast medium) is permitted.~~
  o ~~Other exams for tumor assessment if positive at baseline (e.g. CT or MRI of brain and involved bone~~

~~segments in patients with known brain or bone metastasis) or as clinically indicated (suspected disease progression at new sites)~~

~~After progression to the therapeutic strategy (see 2.5 paragraph) , patients will be followed every 3 months according to clinical practice, and the subsequent lines of therapy and survival data will be registered. For~~ ~~these Ss~~**s**urvival ~~Visits~~**data** phone contacts are acceptable.
~~Every patient will be followed (follow up visits and survival visits) for 1 year after EoCT.~~

25)   Section 5.10 Discontinuation/Withdrawal of Participants from Study Treatment, page 41

Original text

…
- Failure of the treatment strategy because of disease progression (in arm B for patients who receive AI, the strategy failure is defined by disease progression during AI treatment).
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Significant non-compliance with treatment regimen or study requirements
- An AE which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- …

Amended text

…
- Failure of the treatment strategy because of disease progression ~~(in arm B for patients who receive AI, the strategy failure is defined by disease progression during AI treatment).~~
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Significant non-compliance with treatment regimen or study requirements
- An AE which requires discontinuation of ~~the~~**all** study medication**s** or results in inability to continue to comply with study procedures
- …

26)   Section 6 STUDY TREATMENT, page 42

Original text

All the investigational medicinal products used in the clinical trial are authorized for use in ABC, although the concomitant administration of AIs and chemotherapy is not standard practice and is the matter of this study.
<u>Chemotherapy</u> regimens are at the discretion of the treating physician, based also on patient's features and preferences, and must be selected before randomization.
Possible chemotherapy regimen are:
- …based on anthracycline and taxane
- taxane-based (without anthracycline)
- anthracycline-based (without taxane)
- based on capecitabine or other fluoropyrimidines

![Servizio Sanitario Regionale Emilia-Romagna logo]

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Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0

- other

Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard" per each individual agent's prescribing information, with activity documented preferentially through a published phase III clinical trial. Dosage adjustments during treatment are at the discretion of the treating physician, according to clinical practice.

Endocrine therapy:

- non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI, or who relapsed more than 24 months from the end of adjuvant AI
- non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed less than 24 months from the end of adjuvant steroidal AI
- steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed less than 24 months from the end of adjuvant non-steroidal AI

The choice of the AI must be selected before randomization, being one of the stratification factors.

Amended text

All the investigational medicinal products used in the clinical trial are authorized for use in ABC, although the concomitant administration of ~~AIs~~ **endocrine agents** and chemotherapy is not standard practice~~and is the matter of this study~~.

Any cytotoxic or endocrine agent to which a patient was exposed in the adjuvant setting may be administered within this study only if PD occurred at least 12 months after the end of that drug.

Chemotherapy regimens are at the discretion of the treating physician, based also on patient's features and preferences~~, and must be selected before randomization~~.

Possible chemotherapy regimen**s** are:

- based on anthracycline and taxane
- taxane-based (without anthracycline)
- anthracycline-based (without taxane)
- based on capecitabine or other fluoropyrimidines
- other

Chemotherapy regimens and doses should be chosen among those commonly accepted as "standard" per each individual agent's prescribing information~~, with activity documented preferentially through a published phase III clinical trial~~. Dosage adjustments during treatment are at the discretion of the treating physician, according to clinical practice.

Endocrine therapy:

- non-steroidal or steroidal AI, at the discretion of the treating physician, in women not pretreated with an AI~~, or who relapsed more than 24 months from the end of adjuvant AI~~
- non-steroidal AI in women pretreated with a steroidal AI for metastatic disease, or who relapsed ~~less than 24 months from the end of~~**while on** adjuvant steroidal AI
- steroidal AI in women pretreated with a non-steroidal AI for metastatic disease, or who relapsed ~~less than 24 months from the end of~~**on** adjuvant non-steroidal AI
- **fulvestrant in women not pretreated with fulvestrant for advanced disease**

~~The choice of the AI must be selected before randomization, being one of the stratification factors.~~**CDK4/6 inhibitor:**

- **palbociclib**
- **ribociclib**
- **abemaciclib**

27) Section 6.1 Description of Study Treatment, page 43

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

Protocol Code: IRST174.19
Identifier Code: L2P1388
Date and Version: 09/11/2018 – Amendment 1.0
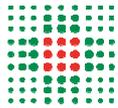
Original text

Possible endocrine treatments are:

- non-steroidal AIs:
    - anastrozole 1 mg daily continuously
    - letrozole 2.5 mg daily continuously
- steroidal AI:
    - exemestane 25 mg daily continuously

…

Anastrozole inhibits … A review of the clinical trial safety database did not reveal evidence of clinically significant interaction in patients treated with anastrozole who also received other commonly prescribed medicinal products.

Allowed chemotherapy regimens are all those registered for metastatic breast cancer including: anthracycline plus taxane, taxane-based (without anthracycline), anthracycline-based (without taxane), capecitabine- (or other fluoropyrimidines)-based, and others.

Amended text

Possible endocrine treatments are:

- non-steroidal AIs:
    - anastrozole 1 mg daily continuously
    - letrozole 2.5 mg daily continuously
- steroidal AI:
    - exemestane 25 mg daily continuously
- **fulvestrant, 500 mg i.m. on days 1, 15, 29 and then every 4 weeks**

…

Anastrozole inhibits … A review of the clinical trial safety database did not reveal evidence of clinically significant interaction in patients treated with anastrozole who also received other commonly prescribed medicinal products.

Fulvestrant metabolism involves multiple transformations, similarly to endogenous steroids, and cytochrome p450 3A4 (CYP3A4) appears involved in its oxidation. Despite that, there are no know interactions with other drugs, including inducers and inhibitors of CYP3A4, and fulvestrant does not inhibit other CYP enzymes.
The CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib are primarily metabolized by CYP3A and sulfotransferase (SULT) enzyme SULT2A1, and are time-dependent inhibitors of CYP3A.
Avoid the concomitant use of CDK4/6 inhibitors with strong CYP3A inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, posaconazole, voriconazole, boceprevir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir, conivaptan, telithromycin, nefazodone; grapefruit or grapefruit juice), which lead to increased plasma exposure of CDK4/6 inhibitors. If coadministration of a strong CYP3A inhibitor cannot be avoided, reduce the dose of CDK4/6 inhibitors.
Avoid concomitant use of CDK4/6 inhibitors with strong CYP3A inducers (e.g., phenytoin, rifampin, carbamazepine, enzalutamide, and St John's Wort), which decrease the plasma exposure of CDK4/6 inhibitors.
The dose of drugs which are CYP3A substrates with a narrow therapeutic index (e.g. midazolam, alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus) may need to be reduced, as CDK4/6 inhibitors may increase their exposure.
The use of ribociclib must be avoided in concomitance with drugs with a known potential to prolong QT such as antiarrhythmic medicines (including, but not limited to amiodarone, disopyramide, procainamide, quinidine and sotalol), and other drugs that are known to prolong the QT interval (including, but not limited to, chloroquine, halofantrine, clarithromycin, haloperidol, methadone, moxifloxacin, bepridil, pimozide and ondansetron).

Allowed chemotherapy regimens are all those registered for metastatic breast cancer including: anthracycline plus

taxane, taxane-based (without anthracycline), anthracycline-based (without taxane), capecitabine- (or other fluoropyrimidines)-based, and others.
See the prescribing information of each drug for further informations.

28) Section 6.5 Concomitant Medication, page 47

Original text

...
Bisphosphonates or a RANK ligand inhibitor may be given according to their product license and routine clinical practice at the investigator's discretion.
Any medication, other than the study medication taken during the study will be recorded in the eCRF.

Amended text

...
Bisphosphonates or a RANK ligand inhibitor may be given according to their product license and routine clinical practice at the investigator's discretion.
Particular attention must be given to concomitant medications in patients receiving CDK4/6 inhibitors, which have e strong potential for drug-drug interactions.
Any medication, other than the study medication taken during the study will be recorded in the eCRF.

29) Section 7.1 Background, page 48

Original text

Any toxicity observed during the course of the study could be managed by dose delay, reduction and/or interruption if deemed appropriate by the Investigator.

Amended text

Any toxicity observed during ~~the course of~~ the study ~~could~~ **can** be managed by dose delay, reduction and/or interruption if deemed appropriate by the Investigator. **Suggested dose modifications are those recommended by the prescribing information leaflets of each drug (in particular for CDK4/6 inhibitors and chemotherapeutic agents) and/or standard dose modifications rules for chemotherapy regimens.**

30) Section 7.2 Monitoring and Toxicity Management, page 42

Original text

Each patient receiving chemotherapy, alone or in combination with AI, will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and reports of AEs reported to the investigator by patients.
Each patient will be assessed periodically for the development of any toxicity as outlined in Section 5. Study Procedures. Toxicity will be assessed according to the NCI CTCAE v4.03.
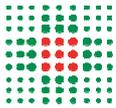
Amended text

Each patient receiving ~~chemotherapy, alone or in combination with AI,~~**at least one administration of study treatments** will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and reports of AEs reported to the investigator by patients.

Each patient will be assessed periodically for the development of any toxicity as outlined in Section 5. Study Procedures. Toxicity will be assessed according to the NCI CTCAE ~~v4~~**v5**.0~~3~~.

31)   Section 8 CORRELATIVE STUDIES, page 48

Original text

With biological correlative studies, we will assess predictors of response to chemotherapy and aromatase inhibitors in archival specimens from the primary tumor or from metastatic samples, as well as in blood samples collected at baseline and then repeated at different time points during therapy.

… Three main pathways are identified as important to this aim: p53, Rb, and p38/JNK MAPK pathways.

Amended text

With biological correlative studies, we will assess predictors of response to chemotherapy and ~~aromatase inhibitors~~**CDK4/6 inhibitors** in archival specimens from the primary tumor or from metastatic samples, as well as in blood samples collected at baseline and then repeated at different time points during therapy.

… Three main pathways are identified as important to this aim: p53, Rb, and p38/JNK MAPK pathways. **The Cyclin D – CDK4/6 – Rb pathway is also crucial for responsiveness to CDK4/6 inhibitors.**

32)   Section 8.1 Background and aim of the study, page 49

Original text

p53 pathway

…

Rb pathway

…

In a retrospective study, p53 status, evaluated by immunohistochemistry, was found to have no predictive value for benefit from adjuvant cyclophosphamide, methotrexate and fluorouracil when considered independently of Rb, but was significantly associated with prognosis in patients with cancers with functioning Rb [Derenzini M, 2009]. In a retrospective study on patients treated with doxorubicin or fluorouracil + mitomycin for locally advanced breast cancer, genetic alterations leading to concomitant inactivation of both the p53 and the Rb pathways predicted resistance towards chemotherapy more strongly than inactivation of either pathway alone [Knappskog S, 2015].

p38 and JNK MAPK pathways

…

Amended text

p53 pathway

…

Rb pathway

…

In a retrospective study on patients treated with doxorubicin or fluorouracil + mitomycin for locally advanced breast cancer, genetic alterations leading to concomitant inactivation of both the p53 and the Rb pathways predicted resistance towards chemotherapy more strongly than inactivation of either pathway alone [Knappskog S, 2015].

The Cyclin D – CDK4/6 – Rb pathway is the target of CDK4/6 inhibitors. ~~While~~The expression of a functioning Rb protein is required for CDK4/6 inhibitors activity [Finn RS, BCR 2009], but loss of Rb function may occur in breast cancers, due to gene mutations or deletion and more frequently due to excessive phosphorylation resulting from activation of upstream pathways or loss of inhibitors. The identification of other response predictors has been elusive until now. Loss of p16 function, due to gene (CDKN2A) mutations or loss, as well as cyclin D1 gene (CCND1) amplification, expected to portend responsiveness from preclinical data [Finn RS, BCR 2009], were not associated with activity in clinical trials [Finn RS, Lancet Oncol 2015]. Predictors of CDK4/6 inhibitors sensitivity have been studied by Gong and colleagues with an antiproliferative screening on a large panel of cancer cell lines, using primarily the CellTiterGlo assay (an ATP-based measure of cell viability) [Gong X, 2017]. CDKN2A loss or mutations confer only intermediate sensitivity to CDK4/6 inhibitors. High levels of cyclins D (D1, 2, or 3) expression are usually present in cancer cell lines responsive to CDK4/6 inhibitors but are not sufficient to confer sensitivity. Several genetic aberrations that lead to activation of D-type cyclins, referred to by the authors as "D-cyclin activating features" (DCAF), have been found to be associated to CDK4/6 inhibitors sensitivity in different cancer cell lines, including CCND1 translocation, CCND1-3 3'UTR loss (leading to a truncated CCND transcript with increased mRNA stability), CCND2 or CCND3 amplification, Kaposi sarcoma virus D-type cyclin (K-cyclin) loss and FBXO31 loss [Gong X, 2017]. CCND1 amplification is associated with high sensitivity to CDK4/6 inhibitors in ER-positive breast cancer cell lines, as well as in thyroid cancer cells, but with relative insensitivity in other cancer cell types (perhaps because of the preminent effect of neighbouring oncogenes co-amplified). Transcriptome data confirm the positive association of CCND2 and CCND3 mRNA levels with sensitivity to CDK4/6 inhibitors, while levels of CCND1 mRNA show inverse correlation with sensitivity. Protein data show that the expression levels of Rb or phosphorylated Rb positively correlate with sensitivity to CDK4/6 inhibitors, while levels of cyclin D1 are associated with relative insensitivity and p16 protein levels are not associated with sensitivity or resistance.

When using the CyQuant assay (a DNA content-based assay and a measure of DNA replication), results usually paralleled those from the CellTiterGlo assay in most cancer cell lines, apart from ER-positive breast cancer lines, that often showed a replication block also in presence of cell viability, highlighting the limited capacity of CDK4/6 inhibitors to completely suppress metabolism and growth. Fulvestrant synergizes with CDK4/6 inhibitors leading to complete metabolic suppression in ER-positive breast cancer cell lines.

Inhibition of CDK4/6 in CDKN2A mutant cells leads, after a transient suppression of Rb phosphorylation, to an increase in CDK2 activity, responsible for the reactivation of Rb phosphorylation and resistance to CDK4/6 inhibitors treatment. This fits with the finding that amplification of CCNE1 (coding for cyclyn E) predicts resistance to CDK4/6 inhibitors. On the contrary, sensitive cells show persistent suppression of Rb phosphorylation and lack of CDK2 activation after exposure to CDK4/6 inhibitors, resulting in cell senescence and apoptosis.

CDK4/6 kinases also phosphorylate factors involved in functions other than cell cycle regulation, affecting cell differentiation, mitochondrial activity, metabolism, antigen processing and presentation and immunogenicity [Klein ME, 2018]. Depending on cell type and transforming event, Rb-proficient cells can undergo quiescence or senescence or apoptosis in response to CDK4/6 inhibitors, but determinants of this different fates are not known. CDK4/6 inhibitors induce oxidative stress with ROS production in ER-positive breast cancer cells, which activate autophagy as a stress response mechanism, and autophagy degrades ROS preventing the induction of senescence. Combining CDK4/6 inhibitors with autophagy inhibitors, such as chloroquine, hydroxychloroquine, and others, induces senescence [Vijayaraghavan S, 2017]. In this study the expression of Rb protein was necessary for responsiveness to CDK4/6 inhibitors, while the expression of a low-molecular weight cyclin E isoform (LMWE), resulting from post-translational cleavage of full-length cyclin E, significantly reduced sensitivity to palbociclib. This highlights the importance of an intact G1/S transition for response to CDK4/6 inhibitors. The predictive value of Rb and LMWE expressions were confirmed on tumor samples from patients treated with fulvestrant + palbociclib for

advanced breast cancer, showing better PFS for Rb+/LMWE- patients, intermediate for Rb+/LMWE+ and shorter PFS for Rb-/LMWE+ patients.

Several gene expression signatures associated with inactivation of the Rb pathway have been developed [Ertel A, Cell Cycle 2010; Chicas A, Cancer Cell 2010; Lara MF, Mol Carcinog 2008; Markey MP, Cancer Res 2002; Markey MP, Oncogene 2007; Witkiewicz AK, Clin Cancer Res 2012; Herschkowitz JI, Breast Cancer Res 2008], and one of them has been shown to discriminate between palbociclib-resistant and sensitive breast cancer cell lines [Malorni L, 2016]. If validated within a clinical trial this could become a further useful biomarker. Finally, serum levels of thymidine kinase, a key enzyme in DNA synthesis with peak expression in the S phase of cell cycle, have been shown to represent a pharmacodynamic marker of CDK4/6 inhibition in patients treated with neoadjuvant palbociclib [Bagegni N, Breast Cancer Res 2017].

p38 and JNK MAPK pathways
…

33)   Section 8.2 Materials and methods, page 61

Original text

We will assess by immunohistochemistry, on formalin fixed paraffin embedded tumor specimens (taken from the metastatic sites when available, and from the primary tumor otherwise), the expression of the following biomarkers: phosphorylated ER, phosphorylated androgen receptor, ER beta, cyclin D1, cyclin E, phosphorylated p38 and JNK mitogen-activated protein kinases, p53, pRb, p16INK4a. This will allow to better characterize the contribution of the AI, over that of chemotherapy, to the activity of combined chemo-endocrine therapy.

The number of CTCs will be measured (by means of the CELLSEARCH® CTC System) at baseline and every 3 months thereafter, and the temporal trends of CTC will be compared between the two treatment arms. In a subgroup of patients, the biological features of CTC will be assessed by means of the lab-on-a-chip system DEPArray, that combines the ability to manipulate and analyze single pure cells in fluorescence microscopy. This will allow to study the expression of epithelial markers (EpCAM, panCK, E-cadherin), mesenchymal markers (vimentin, N-cadherin, O-cadherin), and markers of stemness (ALDH1, CD44, ABCG2).

In further correlative studies we will assess by NGS, on circulating cell-free DNA, the mutational status and copy number variations of genes of the p53 pathway, cell cycle/Rb pathway, p38/JNK MAPKs stress pathways, known to be with some frequency altered in breast cancer.

We will further assess by Nanostring nCounter, on formalin fixed, paraffin embedded samples from the primary tumor or from biopsies of metastatic sites when available, the gene expression profiling of a set of genes.

Tumor tissue samples: an archived formalin-fixed paraffin-embedded (FFPE) block must be collected, from biopsy of a metastatic site when available, or from the primary tumor otherwise. If blocks are unavailable, provide 1 hematoxylin-eosin section and a total of 10 white sections of 5µM placed on positive charge slides from the primary/metastatic tumor sample.

Blood withdrawals:
- Circulating DNA: 10 ml of blood must be collected in a Vacutainer test tube with EDTA (CBC tubes with purple cap), at baseline and every 3 months until therapeutic strategy PD. Gently mix the blood collection tube by inverting 8-10 times immediately after collection. Then centrifuge immediately at 1500 to 2000 g for 15 minutes. This will get approximately 7.5 ml of plasma, and 2.5 ml of cellular pellet. Transfer the sovranatant plasma in aliquots of 1 ml in 2 ml cryovials, and the cellular pellet in aliquots of 1 ml in other cryovials. All cryovials must be stored at -80°C until shipment. Each tube and criovial must clearly indicate:
    - protocol name
    - date of sampling
    - patient personal data or code.
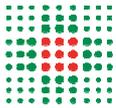
- Circulating Tumor Cells (CTCs): blood samples for the CELLSEARCH® CTCs Test must be collected at baseline, 6-8 weeks after the beginning of study treatment, and 2-4 weeks after the last dose of chemotherapy.
  For specimen collection and preparation see Appendix F. Peripheral blood will be obtained as scheduled in the next section. For CTC count whole blood will be collected into a CellSave® Preservative Tube, processing samples within 96 hours after blood draw. For RNA isolation of CTCs, whole blood will be drawn into 10 ml EDTA tubes, processing samples within 36 hours after venipuncture. Blood draw will be collected as follow:
  - the Cell Save® Tubes will be draw first if multiple tube types are drawn for the protocol;
  - the Cell Save® Tube will be filling until blood flow stops, to meet the required volume for the assays (CTC assay: at least 7.5 ml; CTC enrichment for molecular analysis: at least 7.5 ml);
  - the blood will be mixed IMMEDIATELY by gently inverting the CellSave® Tube 8 times.
  - CellSave® Tubes will be labeled with patient ID, study number and date drawn.
  Samples will be stored and transported at temperatures of 15°-30°C, protecting from extreme temperatures. All sample will be shipped to IOV within 36-72 hours after collection.

Blood samples for circulating DNA must be withdrawn before those for CTCs.

Amended text

We will assess by *immunohistochemistry*, on **formalin fixed paraffin embedded tumor specimens** (taken from the metastatic sites when available, and from the primary tumor otherwise), the expression of the following biomarkers: phosphorylated ER, ~~phosphorylated androgen receptor, ER beta,~~ cyclin D1**-3**, cyclin E, **low-molecular weight cyclin E isoform (LMWE),** phosphorylated p38**,** ~~and~~JNK~~mitogen-activated protein kinases~~, p53, pRb, p16INK4a. ~~This will allow to better characterize the contribution of the AI, over that of chemotherapy, to the activity of combined chemo-endocrine therapy.~~ A *gene expression profiling* **study will be performed on formalin fixed, paraffin embedded samples from the primary tumor or from biopsies of metastatic sites (when available) in a subset of patients (responsive and resistant to CDK4/6 inhibitors and/or to chemotherapy). This will be done with the Nanostring nCounter using the specific breast cancer panel, or by RNA seq, and will include genes of the p53, Rb and p38/JNK pathways.**

The number of **CTCs** will be measured (by means of the CELLSEARCH® CTC System) at baseline, **after 6-8 weeks (2 treatment cycles)** and **at disease progression**~~every 3 months thereafter, and the temporal trends of CTC will be compared between the two treatment arms.~~ **CTC-positive samples will be processed by the micro-dissection microscope (MDM) system to recover the single CTCs and to develop the copy number variation (CNV) and mutation profile analysis for genes of the p53, Rb and p38/JNK pathways at single cell level.** In a subgroup of patients, **enrolled at Meldola, Forlì and Cesena hospitals,** the biological features of CTCs will be assessed by means of the lab-on-a-chip system DEPArray **NxT (Menarini, Silicon Biosystem)**, that combines the ability to manipulate and analyze single pure cells in fluorescence microscopy. This will allow to **characterize the sub-phenotype expressed by CTCs and recover live single cells to evaluate** ~~study~~the expression of epithelial markers (**e.g.** EpCAM, panCK, E-cadherin), mesenchymal markers (**e.g.** vimentin, N-cadherin, O-cadherin) and markers of stemness (**e.g.** ALDH1, CD44, ABCG2**), as well as the expression profile of genes of the p53, Rb and p38/JNK pathways, by RNA-Sequencing analysis.**

In further correlative studies we will assess by NGS, on **circulating cell-free DNA**, the mutational status and ~~copy number variations~~**CNVs** of genes of the p53 pathway, cell cycle/Rb pathway, p38/JNK MAPKs stress pathways, known to be with some frequency altered in breast cancer.
These will include the known genetic predictors: CCND1 translocation, CCND1-3 3'UTR loss, CCND2 or CCND3 amplification, Kaposi sarcoma virus D-type cyclin (K-cyclin) loss and FBXO31 loss.
Serum levels of thymidine kinase 1 (TK1) will be measured by ELISA at different time points during treatment.
~~We will further assess by Nanostring nCounter, on formalin fixed, paraffin embedded samples from the primary tumor or from biopsies of metastatic sites when available, the gene expression profiling of a set of genes.~~

Tumor tissue samples: an archived formalin-fixed paraffin-embedded (FFPE) block must be collected, from biopsy of a metastatic site when available, or from the primary tumor otherwise. If blocks are unavailable, provide 1 hematoxylin-

eosin section and a total of 10 white sections of 5µM placed on positive charge slides from the primary/metastatic tumor sample.

Blood withdrawals:

- **Circulating DNA**: 10 ml of blood must be collected in a Vacutainer test tube with EDTA (CBC tubes with purple cap), at baseline and every 3 months until ~~therapeutic strategy PD~~**disease progression**. Gently mix the blood collection tube by inverting 8-10 times immediately after collection. Then centrifuge immediately at 1500 to 2000 g for 15 minutes. This will get approximately 7.5 ml of plasma, and 2.5 ml of cellular pellet. Transfer the sovranatant plasma in aliquots of 1 ml in 2 ml cryovials, and the cellular pellet in aliquots of 1 ml in other cryovials. All cryovials must be stored at -80°C until shipment. Each tube and criovial must clearly indicate:
    – protocol name
    – date of sampling
    – patient personal data or code.
  Blood samples for circulating DNA must be withdrawn before those for CTCs.
- **Circulating Tumor Cells (CTCs) count (all patients)**: blood samples for the CELLSEARCH® CTCs Test must be collected at baseline, 6-8 weeks after the beginning of study treatment, and ~~2-4 weeks after the last dose of chemotherapy~~**at disease progression.**
  For specimen collection and preparation see Appendix F. ~~Peripheral blood will be obtained as scheduled in the next section.~~ For CTC count whole blood will be collected into a CellSave® Preservative Tube~~, processing samples within 96 hours after blood draw. For RNA isolation of CTCs, whole blood will be drawn into 10 ml EDTA tubes, processing samples within 36 hours after venipuncture.~~Blood draw will be collected as follow:
  - the Cell Save® Tubes will be draw~~n first~~**after other tubes,** if multiple tube types are drawn for the protocol;
  - the Cell Save® Tube will be ~~filling~~**filled** until blood flow stops, to meet the required volume for the assays (CTC assay: at least 7.5 ml~~; CTC enrichment for molecular analysis: at least 7.5 ml~~);
  - the blood will be mixed IMMEDIATELY by gently inverting the CellSave® Tube 8 times.
  - CellSave® Tubes will be labeled with patient ID, study number and date drawn.
  Samples will be stored and transported at temperatures of 15°-30°C, protecting from extreme temperatures. All sample will be shipped to IOV within 36-72 hours after collection.
- **CTC single cell molecular analysis (only patients enrolled at IRST): for RNA expression analysis on CTC, whole blood will be drawn into 10 ml EDTA tubes. For specimen collection and preparation see Appendix G.**
  The EDTA tubes will be filled until blood flow stops, and mixed IMMEDIATELY by gently inverting the EDTA Tube 8-10 times. Samples will be stored and transported at temperatures of 15°-30°C, protecting from extreme temperatures and shipped to IRST laboratory within 2-3 hours after collection.

~~Blood samples for circulating DNA must be withdrawn before those for CTCs.~~

34) Section 8.3 Samples labelling and shipment, page 63

Original text

The blood samples for circulating markers (other than CTCs) are shipped to the IRST IRCCS Biological laboratory in thermo-boxes containing sufficient dry ice. Dry ice will be provided by IRST IRCCS to each center, through the Dry ice company.
The Tumor tissue samples are ambient shipped to the IRST IRCCS Biological laboratory.
…
After receiving the samples, the IRST IRCCS laboratory staff will complete the "Sample Arrival Form" (see specific guide lines), in order to maintain rigorous confidentiality standards.

The shipment of blood samples for CTCs evaluation must be announced via e-mail (rita.zamarchi@unipd.it) including date of shipment and shipping bill number.
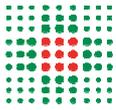
Every blood tube will be labelled with the following information: Study code IRST174.19, site number, subject number, type of sample, timing and date of collection, in order to maintain rigorous confidentiality standards.
...

Amended text

The blood samples for circulating ~~markers (other than CTCs)~~**DNA** are shipped to the IRST IRCCS Biological laboratory in thermo-boxes containing sufficient dry ice. Dry ice will be provided by IRST IRCCS to each center, through the Dry ice company.
The CTC blood draws in EDTA tubes from Forlì and Cesena hospitals, are shipped to the IRST IRCCS Biological laboratory in thermo-boxes at room temperature.
The Tumor tissue samples are ambient shipped to the IRST IRCCS Biological laboratory.
...
After receiving the samples, the IRST IRCCS laboratory staff will complete the "Sample Arrival Form" (see specific guide lines), in order to maintain rigorous confidentiality standards.

CTC count will be assessed with the CELLSEARCH® CTCs Test at the Laboratory of Istituto Oncologico Veneto (Dr.ssa Rita Zamarchi).
The shipment of blood samples for CTCs **count (Cell Save® Tubes)**~~evaluation~~ must be announced via e-mail (rita.zamarchi@unipd.it) including date of shipment and shipping bill number.
Every **Cell Save® Tube** ~~blood tube~~will be labelled with the following information: Study code IRST174.19, site number, subject number, type of sample, timing and date of collection, in order to maintain rigorous confidentiality standards.
...

35) Section 10 SAFETY REPORTING, page 67

Original text

Analyses will be performed for all patients having received at least one dose of study drug. CTCAE Version 4.03 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant.
…

Amended text

Analyses will be performed for all patients having received at least one dose of study drug. CTCAE Version ~~4~~5.0~~3~~ should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant.
…

36) Section 10.2 Reporting Procedures for All AEs, page 70

Original text

...
The following information will be recorded: description, date of onset and end date, severity (according to CTCAE version 4.03), assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.
...

Amended text

...
The following information will be recorded: description, date of onset and end date, severity (according to CTCAE version ~~45~~.0~~3~~), assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.
...

37) Section 11.1 Study Design/Endpoints, page 73

Original text

This study is a randomized, multicenter, two-arm, open label trial designed to compare the efficacy and safety of concomitant chemotherapy and AI versus chemotherapy followed by AI in patients with locally advanced or metastatic luminal breast cancer.

Amended text

This study is a ~~randomized,~~ multicenter, two-arm, open label **group sequential response adaptive randomized phase 2** trial designed to compare the efficacy and safety of **a combination of chemotherapy plus endocrine therapy (AI or fulvestrant) versus a cyclin-dependent kinase 4/6 inhibitor plus endocrine therapy (AI or fulvestrant) as treatment for patients with locally advanced or metastatic HR-positive, HER2-negative breast cancer with doubtful endocrine sensitivity or primary endocrine resistance.**~~concomitant chemotherapy and AI versus chemotherapy followed by AI in patients with locally advanced or metastatic luminal breast cancer.~~

38) Section 11.2 Sample Size, Accrual Rate and Study Duration, page 73

Original text

The primary endpoint is PFS, and the underlying statistical hypothesis is that the concomitant treatment will improve PFS compared with the sequential use of the two treatments.
Based on results from large randomized trials of modern chemotherapy regimens, a median PFS of 6 months is expected for patients undergoing first-line treatment for metastatic breast cancer (standard arm, λ_1=0.077). In order to detect an increase of PFS of 3 months (from 6 to 9 months, λ_2=0.1155, hazard ratio 0.67) in patients receiving concomitant chemo-endocrine therapy, with a two-tail logrank test at 90% power and 5% significance level, 150 patients must be enrolled in each arm (300 patients overall, with 256 expected events) in a period of 24 months (accrual rate: 12.5 patients/month), with further 12 months of follow up.
All patients fulfilling the eligibility criteria will be randomized by the Biostatistics and Clinical Trial Unit of the CC, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST). Patients will be randomized on a 1:1 allocation rate. No blinding is planned.
The study duration will be 4 years; 24 months of accrual and 1 year of follow-up on the last participant enrolled.

Amended text

The primary endpoint is PFS, and the underlying statistical hypothesis is that the ~~concomitant~~**combination of chemotherapy plus endocrine therapy (AI or fulvestrant) (i.e. arm B)** ~~treatment~~will improve PFS compared with **concomitant cyclin-dependent Kinase 4/6 (CDK4/6) inhibitor (palbociclib, ribociclib or abemaciclib) plus endocrine therapy (AI or fulvestrant)**~~the sequential use of the two treatments~~.
Assuming that the survival times follow an exponential distribution parameterized in terms of its expected value, the statistical hypothesis system is $H_0: \theta_A = \theta_B$; $H_1: \theta_B > \theta_A$ at $\alpha = 0.10$ significance level.

A maximum sample size of 150 patients will be recruited in a 23 month period for an estimated accrual rate of around 6.5 patients per month; they will be monitored for a 16 month follow-up.

Initially, the patients will be randomly assigned according to block randomization (with blocks of size two) until two events (i.e. first disease progression or death whichever occurs first) are experienced in both arms. At this point, the allocation probabilities will be computed for the first time according to the Doubly-adaptive Biased Coin Design (DBCD) [Hu 2004] for time-to-event data [Zhang 2007] targeting the Neyman allocation rule (i.e. the one minimizing the total number of patients $n = n_A + n_B$). This randomized response adaptive procedure skews the allocation probabilities towards the arm with a longer estimated PFS allowing for a sensible ethical gain. Subsequently, these probabilities will be updated after 105 and 128 patients enter the study. At these time points, interim analyses will be performed in order to test the null hypothesis of equality of survival times [Zhu 2010]: whether will it be rejected the study is stopped and treatment B declared superior to A. In order to preserve the nominal significance level for the final analysis, Lan and DeMets α-spending function [Lan KG 1983] will be adopted for opportunely correcting the intermediate significance levels. The introduction of the interim analyses further increases the ethical component of the design ensuring that no more than necessary patients will be enrolled. Table 1 shows the results of Monte Carlo simulations carried out under a growing median PFS in arm B –i.e. $_{med}\theta_B$ ranges from eight to 14 months– against a fixed eight month PFS in arm A in order to evaluate the operating characteristics with a maximum sample size of 150 patients. Under the null hypothesis (first row of Table 1) both the usual completely randomized design (CR) and the group sequential DBCD (G-DBCD) show a good control of type-I error rates. Further simulations, not displayed here for sake of brevity, confirmed that both designs maintain this paramount capability even for higher sample sizes and/or longer trial recruitment and duration. Furthermore, G-DBCD expected sample size (ESS) is close to the 150 patients CR fixed one while a good amount of patients is adaptively allocated ($\tau = 0.779$) in an almost balanced way ($\pi_B = 0.498$). This means that under the null hypothesis of equal PFSs, neither arm is preferred.

Increasing the median PFS of treatment B, allows to appreciate the considerable gain in terms of power induced by G-DBCD which allows the log-rank test to far better detect a longer PFS in arm B than under CR. Furthermore this ability is coupled with a progressively lower expected sample size compared to the fixed CR one. Less patients are adaptively assigned as the increase of $_{med}\theta_B$ results in a delay of the starting of the response adaptive randomization procedure since in average it takes more time to observe two events in arm B. On the other hand, the allocation proportion are increasingly skewed towards treatment B as it progressively shows a longer PFS.

Let us a now consider a median eight month PFS for arm A (Paloma 3 Study, supplementary material of Turner NC, NEJM 2018) and a 12 month one for arm B –i.e. $_{med}\theta_A = 8$ and $_{med}\theta_B = 12$. Under this more likely scenario, the log-rank test coupled with G-DBCD is able to detect a four month median PFS increment in arm B with a considerably higher simulated power than under CR design: 0.911 versus 0.717, respectively. In addition, when compared to the fixed 150 patient sample size of the CR design, the G-DBCD shows a reduction of almost 29 patients in the expected sample size (ESS) due to the introduction of interim analyses allowing for early stopping for efficacy. This means that on average around 19% less individuals will be enrolled in the trial, stressing the considerable ethical gain of the proposed strategy. Concurrently, for the log-rank test to achieve the same power (0.911) as under G-DBCD analytical results shows that under CR design on average it needs around 184 total patients –i.e. an increase of around 52% patients with respect to the 121.259 ESS. Finally, under G-DBCD, on average, slightly more than 56% of the ESS is allocated to arm B (π_B): a considerable ethical gain is again appreciable.

Table 2. Results of the Monte Carlo simulation for log-rank test coupled with the complete randomization (CR) and group sequential DBCD (G-DBCD) assuming a median PFS of eight months for arm A and an increasing one for the experimental one ($_{med}\theta_B$): power of the two strategies and expected sample size (ESS), allocations proportions to the experimental (π_B) and percentages of patients adaptively allocated to arm B (τ) under G-DBCD.

$_{med}\theta_B$	power		ESS	π_B	τ
	CR	G-DBCD			

8.0	0.084	0.089	147.014	0.498	0.779
9.0	0.205	0.458	138.317	0.509	0.758
10.0	0.384	0.741	129.897	0.529	0.732
11.0	0.564	0.841	125.304	0.547	0.713
12.0	0.717	0.911	121.259	0.563	0.695
13.0	0.829	0.948	117.939	0.580	0.678
14.0	0.908	0.972	115.130	0.595	0.662

Figure 2. Power of the nonparametric test under complete randomization (CR) and group sequential DBCD (G-DBCD).

All patients fulfilling the eligibility criteria will be randomized by the Biostatistics and Clinical Trial Unit of the CC, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST). No blinding is planned.
The study duration will be 39 months; 23 months of accrual and 16 months of follow-up on the last participant enrolled.

39) Section 11.3 Stratification Factors, page 77

Original text

Patients will be stratified by: type of chemotherapy regimen (5 levels: based on anthracycline and taxane, taxane-based without anthracycline, anthracycline-based without taxane, based on capecitabine or other fluoropyrimidines, other), type of AI (2 levels: non-steroidal, steroidal), previous endocrine therapy for ABC (2 levels: pre-treated, not pre-treated).
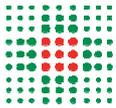
Amended text

~~Patients will be stratified by: type of chemotherapy regimen (5 levels: based on anthracycline and taxane, taxane-based without anthracycline, anthracycline-based without taxane, based on capecitabine or other fluoropyrimidines, other), type of AI (2 levels: non-steroidal, steroidal), previous endocrine therapy for ABC (2 levels: pre-treated, not pre-treated).~~**No stratification factor will be considered in the primary endpoint analysis.**

40) Section 11.4 Analysis of Primary Endpoints, page 77

Original text

…
Comparisons between the two treatment arms will be performed using the stratified log rank test, at a significance level of 5%. Unadjusted and adjusted hazard ratios (HR) will be calculated using Cox proportional-hazard models, including or not the main know prognostic and predictive factors. Two-sided 95% CI for each HR will be provided.

Amended text

…
Comparisons between the two treatment arms will be performed using the ~~stratified~~log rank test, at a significance level of ~~5~~10%. Unadjusted and adjusted hazard ratios (HR) will be calculated using Cox proportional-hazard models, including or not the main know prognostic and predictive factors. Two-sided 95% CI for each HR will be provided.

41) Section 11.6 Interim Analysis, page 78

Original text

No interim analysis is planned.

Amended text

~~No interim analysis is planned.~~
Two interim analyses are planned after 105 and 128 patients are enrolled (i.e. after 70% and 85% of the maximum sample size, respectively). At each halt the PFS equality between the two arms against the superiority of arm B will be inferred by means of the log-rank test. The family wise error rate will be controlled by means of Lan and DeMets α-spending function [Lan KG 1983] in order to preserve the nominal $\alpha = 0.10$ significance level toward the final analysis.

42) Section 19 REFERENCES, page 87

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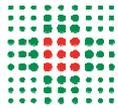
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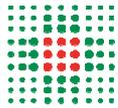
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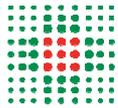
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43)   APPENDIX B, page 112

Original text

The manual is available here:
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Amended text

The manual is available here:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.p
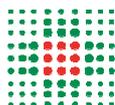
df

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

44)   APPENDIX C, page 114

Original text

Time	Baseline	Cycle 1	Cycle 2	Cycle 3	Cycle n	End of treatment	Follow up visits[18]
Day	-28 to 0	1	1	1	1		
...							
Serum Hepatitis assessment[8]	X						
...							
Blood samples for CTCs[12]	X			X	X		
...							

Treatment administration should begin ≤72 hours of the date of randomization.

...

8. Serum Hepatitis assessment according to institutional practice (e.g. Hepatitis B surface antigen and Hepatitis C virus antibody).

...

17. Assessment of adverse event (CTCAE version 4.03, Appendix C).

...

Amended text

Time	Baseline	Cycle 1	Cycle 2	Cycle 3	Cycle n	End of treatment	Follow up visits[18]
Day	-28 to 0	1	1	1	1		
...							
~~Serum Hepatitis assessment[8]~~	~~X~~						
...							
Blood samples for CTCs12	X			X	~~X~~	X	
...							

Treatment administration should begin ≤72 hours of the date of randomization.

...

8. Serum Hepatitis assessment according to institutional practice (e.g. Hepatitis B surface antigen and Hepatitis C virus antibody).

...

16. Assessment of adverse event (CTCAE version ~~4~~5.0~~3~~, Appendix **B**~~C~~).

...
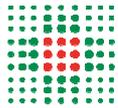
45)   APPENDIX F, page 118

Original text

Modalità di esecuzione del prelievo di sangue

Per la conta delle CTCs il prelievo di sangue intero (10 ml) deve essere necessariamente eseguito in provette CellSave® Preservative Tubes.

1. Il campione iniziale deve essere raccolto prima di avviare un regime terapeutico. I campioni successivi devono essere prelevati secondo il calendario previsto per i due bracci di studio: allo screening, dopo 6-8 settimane dall'avvio del trattamento, e dopo 2-4 settimane dall'ultima somministrazione di chemioterapia.
   ...

Amended text

...
Modalità di esecuzione del prelievo di sangue

Per la conta delle CTCs il prelievo di sangue intero (10 ml) deve essere necessariamente eseguito in **provette CellSave® Preservative Tubes**.

1. Il campione iniziale deve essere raccolto prima di avviare un regime terapeutico. I campioni successivi devono essere prelevati secondo il calendario previsto per i due bracci di studi~~o: allo screening, dopo 6-8 settimane dall'avvio del trattamento, e dopo 2-4 settimane dall'ultima somministrazione di chemioterapia~~.
   ...

46)   APPENDIX G , page 119

Original text

This appendix was not present.

Amended text

PROCEDURA PER L'ESECUZIONE DEL PRELIEVO PER L'ARRICCHIMENTO DELLE CELLULE TUMORALI CIRCOLANTI (CTCs) VITALI E PER L'ANALISI DI ESPRESSIONE GENICA SU SINGOLA CELLULA

L'analisi consente la caratterizzazione delle CTC presenti nel sangue intero.

**SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA**
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

Modalità di esecuzione del prelievo di sangue

Il prelievo di sangue intero (10 ml) deve essere necessariamente eseguito in provette EDTA Tubes.

1. **Il campione iniziale deve essere raccolto prima di avviare un regime terapeutico. I campioni successivi devono essere prelevati secondo il calendario previsto per i due bracci di studio.**
2. **Il prelievo di sangue intero deve essere eseguito in modo asettico mediante venipuntura o porta venosa esclusivamente in provette EDTA Tubes da 9-10 ml.**
3. **E' necessario riempire la provetta fino all'arresto del flusso sanguigno, per assicurare il corretto rapporto tra campione e mix di anticoagulante e conservante. E' importante miscelare immediatamente capovolgendo delicatamente la provetta otto volte. Il capovolgimento della provetta impedisce la formazione di coaguli. Una miscelazione effettuata in modo inadeguato o ritardato può portare a risultati errati.**
4. **I campioni raccolti devono pervenire al laboratorio entro 3-4 ore MAX dal prelievo. Possono essere conservati e trasportati a temperatura ambiente (da 15 a 30°C). Non refrigerare.**

E' bene preavvisare per e-mail (massimiliano.bonafe@irst.emr.it) la spedizione dei campioni.
I campioni vanno inviati (orario accettazione: lun-ven, 8.30-15.00) a:

Dr. Massimiliano Bonafèi
c/o Laboratorio di Bioscienze,
IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST),
Via Maroncelli 40,
47014 Meldola (FC).
E-mail: massimiliano.bonafe@irst.emr.it
Phone: +39 0543 739905, Fax: +39 0543 739221

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

MEDICAL HISTORY [CHENDO] - CHENDO 1.0

Section Title: HISTORY OTHER CANCERS
Instructions: Answer all required questions (marked with *)

1)
Did the patient have any other
cancer? *

◯ Yes ◯
No

**As per protocol: Prior non-breast malignancy should have
been treated with curative intent and disease free for
at least 3 years (except for adequately controlled basal
cell carcinoma of the skin, carcinoma in situ of the cervix,
in situ carcinoma of the bladder). If this criterium is not
fulfilled, please, contact the Coordinating Center.**

HISTORY OTHER CANCERS	
2) Type of cancer	3) Date of diagnosis

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: **PREVIOUS SURGERY**
Instructions: Answer all required questions (marked with *)

1) Did the patient receive previous   ◯ Yes ◯ No
    surgical interventions? *

PREVIOUS SURGERY		
2) Type of surgery	**3) Setting**	**4) Date of surgery**
	◯ Select… ◯ Curative ◯ Palliative	

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: PREVIOUS THERAPY
Instructions: Answer all required questions (marked with *)

1) Did the patient receive previous oncological therapy? * ○ Yes ○ No

PREVIOUS THERAPY					
2) Therapy	**3) Setting**	**4) Number of cycles**	**5) Start date**	**6) End date**	**7) Reason for interruption**
	○ Select… ○ Neoadjuvant ○ Adjuvant ○ Advanced	○ Select… ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10 ○ NA ○ Unknown			○ Select… ○ Toxicity ○ Progression/Relapse ○ Refusal ○ Treatment completion ○ Other ○ Unknown

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: PREVIOUS RADIOTHERAPY

Instructions: Answer all required questions (marked with *)

1)
Did the patient receive previous radiotherapy? *

○ Yes ○ No

As per protocol, previous radiotherapy is allowed if not performed within 4 weeks from study entry. If this criterium is not fulfilled, please, contact the Coordinating Center.

PREVIOUS RADIOTHERAPY			
2) Irradiation site	3) Setting	4) Start date	5) End date
	○ Select… ○ Adjuvant ○ Curative ○ Palliative		

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

PHYSICAL EXAMINATION [CHENDO] - CHENDO 1.0

Section Title: PHYSICAL EXAMINATION
Instructions: Answer all required questions (marked with *)

1) Was physical examination performed? *   ◯ Yes ◯ No

2) Date of physical examination *

3) ECOG performance status *
◯ Select…
◯ 0
◯ 1
◯ 2
◯ 3
◯ 4

4) Blood pressure max *   (mmHg)

5) Blood pressure min *   (mmHg)

6) Pulse rate *   (/min)

7) Body temperature *   (°C)

8) Weight *   (kg)

9) Height *   (cm)

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

PREGNANCY TEST [CHENDO] - CHENDO 1.0

Section Title: PREGNANCY TEST
Instructions: Answer all required questions (marked with *)

1) Was pregnancy test performed? *   ○ Yes ○ No ○ NA

2) Date of pregnancy test *

3) HCG *   ○ Select…

                                 ○ positive

                                 ○ negative

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

QUESTIONNAIRES [CHENDO] - CHENDO 1.0

Section Title: QUESTIONNAIRES
Instructions: Answer all required questions (marked with *)

1) Questionnaire Timepoint *   ◯ Select…

◯ Screening

◯ Treatment phase

◯ EoT

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: EORTC-QLQ-C30

Instructions: Answer all required questions (marked with *)

Was EORTC QLQ-C30 questionnaire filled? *
   ○ Yes ○ No

Date EORTC QLQ-C30 questionnaire filled *

1. Ha difficoltà nel fare lavori faticosi, come portare una borsa della spesa pesante o una valigia?
- ○ Select...
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

2. Ha difficoltà nel fare una lunga passeggiata?
- ○ Select...
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

3. Ha difficoltà nel fare una breve passeggiata fuori casa?
- ○ Select...
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

4. Ha bisogno di stare a letto o su una sedia durante il giorno?
- ○ Select...
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

5. Ha bisogno di aiuto per mangiare, vestirsi, lavarsi o andare in bagno?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

Durante gli ultimi sette giorni:

6. Ha avuto limitazioni nel fare il Suo lavoro o i lavori di casa?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

7. Ha avuto limitazioni nel praticare i Suoi passatempi-hobby o altre attività di divertimento o svago?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

8. Le è mancato il fiato?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

9. Ha avuto dolore?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

10. Ha avuto bisogno di riposo?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

11. Ha avuto difficoltà a dormire?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

12. Ha sentito debolezza?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

13. Le è mancato l'appetito?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

14. Ha avuto un senso di nausea?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

15. Ha vomitato?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

16. Ha avuto problemi di stitichezza?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

17. Ha avuto problemi di diarrea?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

18. Ha sentito stanchezza?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

19. Il dolore ha interferito con le Sue attività quotidiane?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

20. Ha avuto difficoltà a concentrarsi su cose come leggere un giornale o guardare la televisione?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

21. Si è sentito(a) teso(a)?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

22. Ha avuto preoccupazioni?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

23. Ha avuto manifestazioni di irritabilità?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

24. Ha avvertito uno stato di depressione?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

25. Ha avuto difficoltà a ricordare le cose?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

26. Le Sue condizioni fisiche o il Suo trattamento medico hanno interferito con la Sua vita familiare?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

27. Le Sue condizioni fisiche o il Suo trattamento medico hanno interferito con le Sue attività sociali?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

28. Le Sue condizioni fisiche o il Suo trattamento medico Le hanno causato difficoltà finanziarie?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

29. Come valuterebbe in generale la Sua salute durante gli ultimi sette giorni?

○ Select...

○ 1-pessima

○ 2

○ 3

○ 4

○ 5

○ 6

○ 7-Ottima

○ Not done

30. Come valuterebbe in generale la Sua qualità di vita durante gli ultimi sette giorni?

○ Select...

○ 1-pessima

○ 2

○ 3

○ 4

○ 5

○ 6

○ 7-Ottima

○ Not done

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: EORTC-QLQ-BR23

Instructions: Answer all required questions (marked with *)

Was EORTC QLQ-BR23
questionnaire filled? *   ○ Yes ○ No

Date EORTC QLQ-BR23
questionnaire filled *

Durante gli ultimi sette giorni:

31. Ha avuto la bocca asciutta?   ○ Select...
                                             ○ No
                                             ○ Un pò
                                             ○ Parecchio
                                             ○ Moltissimo
                                             ○ Not done

32. Il cibo e le bevande hanno
avuto un sapore diverso dal solito?   ○ Select...
                                             ○ No
                                             ○ Un pò
                                             ○ Parecchio
                                             ○ Moltissimo
                                             ○ Not done

33. I Suoi occhi sono stati dolenti,
irritati o hanno lacrimato?   ○ Select...
                                             ○ No
                                             ○ Un pò
                                             ○ Parecchio
                                             ○ Moltissimo
                                             ○ Not done

34. Ha perso dei capelli?   ○ Select...
                                             ○ No
                                             ○ Un pò
                                             ○ Parecchio
                                             ○ Moltissimo
                                             ○ Not done

35. Risponda solo se ha perso dei capelli: in caso affermativo, la caduta dei capelli l'ha turbata?

- ○ Select…
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

36. Si è sentita male o poco bene?

- ○ Select…
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

37. Ha avuto vampate di calore?

- ○ Select…
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

38.Ha avuto mal di testa?

- ○ Select…
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

39. Si è sentita fisicamente meno attraente come conseguenza della Sua malattia o del trattamento?

- ○ Select…
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

40.Si è sentita meno femminile come conseguenza della Sua malattia o del trattamento?

- ○ Select…
- ○ No
- ○ Un pò
- ○ Parecchio
- ○ Moltissimo
- ○ Not done

41. Ha trovato qualche difficoltà a guardarsi nuda?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

42. Si è sentita insoddisfatta del Suo corpo?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

43. Ha avuto preoccupazioni per la Sua salute nel futuro?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

Nel corso delle ultime 4 settimane:

44. Si è sentita interessata al sesso?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

45. In che misura è stata sessualmente attiva (con o senza rapporto sessuale completo)?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

46. Risponda a questa domanda solo se sessualmente attiva: è stato piacevole il sesso per Lei?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

Durante gli ultimi sette giorni:

47. Ha avuto dolori al braccio o alla spalla?

○ Select...
○ No
○ Un pò
○ Parecchio
○ Moltissimo
○ Not done

48. Ha avuto il braccio o la mano gonfi?

○ Select...
○ No
○ Un pò
○ Parecchio
○ Moltissimo
○ Not done

49. Ha avuto difficoltà ad alzare il braccio o a muoverlo lateralmente?

○ Select...
○ No
○ Un pò
○ Parecchio
○ Moltissimo
○ Not done

50. Ha avuto dolore nell'area del seno ammalato?

○ Select...
○ No
○ Un pò
○ Parecchio
○ Moltissimo
○ Not done

51. L'area del seno ammalato è stata gonfia?

○ Select...
○ No
○ Un pò
○ Parecchio
○ Moltissimo
○ Not done

52. L'area del seno ammalato è stata ipersensibile?

○ Select...
○ No
○ Un pò
○ Parecchio
○ Moltissimo
○ Not done

53. Ha avuto problemi dermatologici (di pelle) nell'area del seno ammalato (es.prurito, pelle secca, pelle che si squama)?

○ Select...

○ No

○ Un pò

○ Parecchio

○ Moltissimo

○ Not done

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

RANDOMIZATION [CHENDO] - CHENDO 1.0

Section Title: RANDOMIZATION
Instructions: Answer all required questions (marked with *)

Site
*

Treatment   Randomization date

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

SEROLOGICAL LAB EXAM [CHENDO] - CHENDO 1.0

Section Title: SEROLOGICAL LAB EXAM
Instructions: Answer all required questions (marked with *)

1) Did the patient perform serological   ○ Select...
   lab exams? *
                                           ○ Yes

                                           ○ No

2) Date blood sample taken *

3) HCV *   ○ Select...

                                           ○ Negative

                                           ○ Positive

                                           ○ Not done

4) HBV *   ○ Select...

                                           ○ Negative

                                           ○ Positive

                                           ○ Not done

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

STUDY TREATMENT [CHENDO] - CHENDO 2.0

Section Title: CHEMOTHERAPY
Instructions: Answer all required questions (marked with *)

1) Did the patient take chemotherapy? *

- ○ Select...
- ○ Yes
- ○ No
- ○ Not applicable

CHEMOTHERAPY

2) Chemotherapy	3) Start date	4) End date	5) Daily Dose Administered	6) Unit	7) Total daily dose Administered	8) Unit	9) Route	10) If "other", please specify	11) Dose modification?	12) Reason modification	13) Specify AE nr.	14) Other specify
○ Select... ○ Docetaxel ○ Paclitaxel ○ Protein-bound paclitaxel ○ Epirubricin ○ Doxorubicin ○ Pegylated liposomal doxorubicin ○ Fluorouracile ○ Capecitabine ○ Cyclophosphamide ○ Methotrexate ○ Cisplatin ○ Carboplatin ○ Eribulin ○ Gemcitabine ○ Vinorelbine ○ Ixabepilone ○ Other				○ Select... ○ mg/mq ○ mg/kg		○ Select... ○ mg ○ g	○ Select... ○ Oral ○ Intravenous ○ Subcutaneous ○ Intramuscolar ○ Other		○ Select... ○ Dose not changed ○ Dose delayed ○ Dose witheld ○ Dose reduced ○ Drug permanently discontinued	○ Select... ○ Adverse Event ○ Disease progression ○ Achievement of maximum response ○ Uncompliance ○ Physician decision ○ Patient decision ○ Other		

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: ENDOCRINE THERAPY
Instructions: Answer all required questions (marked with *)

1) Did the patient take Endocrine therapy? *

    ○ Select…

    ○ Yes

    ○ No

    ○ Not applicable

ENDOCRINE THERAPY

2) Endocrine therapy	3) Start date	4) End date	5) Total daily dose Administered	6) Dose modification?	7) Reason modification	8) Specify AE nr.	9) Other specify
○ Select… ○ Exemestane ○ Anastrozole ○ Letrozole			(mg)	○ Select… ○ Dose not changed ○ Dose delayed ○ Dose witheld ○ Dose reduced ○ Drug permanently discontinued	○ Select… ○ Adverse Event ○ Disease progression ○ Uncompliance ○ Physician decision ○ Patient decision ○ Other		

Protocol ID: _____　　　　　Study Subject ID:_____

Study Name: _____　　　　　Interviewer Name:_____

Site: _____　　　　　　　　　Interview Date:_____

Event Name: _____

Event Date: _____

SURVIVAL [CHENDO] - CHENDO 1.0

Section Title: MOST RECENT CONTACT
Instructions: Answer all required questions (marked with *)

Please complete SURVIVAL FORM every 3 months after Disease progression for 1 year or until Death or Lost to follow-up.

1) Date of most recent visit or phone contact *

2) Is the patient still alive? *　　　　　○ Select…

　　　　　　　　　　　　　　　　　　○ Yes

　　　　　　　　　　　　　　　　　　○ No

　　　　　　　　　　　　　　　　　　○ UNK (Lost to Follow Up)

3) Has the patient received any anticancer therapy? *　　　○ Select…

　　　　　　　　　　　　　　　　　　○ Yes

　　　　　　　　　　　　　　　　　　○ No

4) Anticancer therapy start date *

5) Name of therapy *

6) Date of death *

7) Cause of death *　　　　　　　　　○ Select…

　　　　　　　　　　　　　　　　　　○ Tumor

　　　　　　　　　　　　　　　　　　○ Toxicity

　　　　　　　　　　　　　　　　　　○ Concomitant disease present at baseline

　　　　　　　　　　　　　　　　　　○ Other

　　　　　　　　　　　　　　　　　　○ UNK

8) If Toxicity, please specify *

9) If Concomitant disease present at baseline, please specify *

10) If Other, please specify *

11) Comments

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

TUMOR ASSESSMENT [CHENDO] - CHENDO 1.0

Section Title: TUMOR ASSESSMENT
Instructions: Answer all required questions (marked with *)

1) Was tumor assessment performed? ○ Yes ○ No
   *

2) Chest *   ○ Select...
                                    ○ performed
                                    ○ not performed

3) Method of assessment *   ○ Select...   4) Date of assessment *
                                    ○ CT scan
                                    ○ MRI

5) Abdomen *   ○ Select...
                                    ○ performed
                                    ○ not performed

6) Method of assessment *   ○ Select...   7) Date of assessment *
                                    ○ CT scan
                                    ○ MRI

8) Pelvis *   ○ Select...
                                    ○ performed
                                    ○ not performed

9) Method of assessment *   ○ Select...   10) Date of assessment *
                                    ○ CT scan
                                    ○ MRI

11) Brain *   ○ Select...
                                    ○ performed
                                    ○ not performed

12) Method of assessment *   ○ Select...   13) Date of assessment *
                                    ○ CT scan
                                    ○ MRI

14) Bone *   ○ Select...
                                    ○ performed
                                    ○ not performed

15) Method of assessment *   ○ Select...   16) Date of assessment *
                                    ○ CT scan
                                    ○ MRI

17) Other assessments (if clinically ○ Performed ○ Not performed
    indicated) *

18) Type of assessment *   ○ Select...
                                  ○ X-ray
                                  ○ Ultrasound
                                  ○ 18F-FDG PET/CT
                                  ○ Physical exam
                                  ○ Bone scan
                                  ○ Other

19) Specify *   20) Date of assessment *

18) Type of assessment *   ○ Select...
                                  ○ X-ray
                                  ○ Ultrasound
                                  ○ 18F-FDG PET/CT
                                  ○ Physical exam
                                  ○ Bone scan
                                  ○ Other

Protocol ID: _____　　　　　Study Subject ID:_____

Study Name: _____　　　　　Interviewer Name:_____

Site: _____　　　　　　　　　Interview Date:_____

Event Name: _____

Event Date: _____

TUMOR EVALUATION - SCREENING [CHENDO] - CHENDO 1.0

Section Title: TUMOR EVALUATION
Instructions: Answer all required questions (marked with *)

1) Did patient perform tumor
   evaluation? *　　　　　　　○ Yes　○ No

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: TARGET LESIONS

Instructions: Answer all required questions (marked with *)

1)
Has patient target lesions? *

○ Select…

○ Yes

○ No

○ Not applicable

Please complete table below only if patient performed tumor evaluation for target lesions

TARGET LESIONS

2) Location code	3) Description	4) Date of assessment	5) Method of measurement	6) Measurement of Target lesion
			○ Select… ○ CT-scan ○ MRI ○ PET ○ Physical Exam ○ Scintigraphy ○ Spiral CT ○ Ultrasonography ○ X-ray ○ Other	(mm)

○ Select...				
○ Adrenal				
○ Ascites				
○ Bone				
○ Bone marrow				
○ Breast				
○ Cervix				
○ CNS				
○ Colon				
○ Esophagus				
○ Head/neck				
○ Kidney				
○ Liver				
○ Lung				
○ Lymph nodes				
○ Omentum				
○ Oral cavity				
○ Ovary				
○ Pancreas				
○ Peritoneum				
○ Pleura				
○ Pleural effusion				
○ Prostate				
○ Rectum				
○ Retroperitoneal mass				
○ Skin				
○ Small intestine				
○ Spinal marrow				
○ Spleen				
○ Stomach				
○ Testis				
○ Uterus				
○ Other				

7) SUM   (mm)

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: NON TARGET LESIONS

Instructions: Answer all required questions (marked with *)

1)
Has patient non target lesions? *   ○ Select…   Please complete table below only if patient performed tumor evaluation for non target lesions

○ Yes

○ No

○ Not applicable

NON TARGET LESIONS			
2) Location code (site)	**3) Description**	**4) Date of assessment**	**5) Method of measurement**
			○ Select…
			○ CT-scan
			○ MRI
			○ PET
			○ Physical Exam
			○ Scintigraphy
			○ Spiral CT
			○ Ultrasonography
			○ X-ray
			○ Other

- ○ Select...
- ○ Adrenal
- ○ Ascites
- ○ Bone
- ○ Bone marrow
- ○ Breast
- ○ Cervix
- ○ CNS
- ○ Colon
- ○ Esophagus
- ○ Head/neck
- ○ Kidney
- ○ Liver
- ○ Lung
- ○ Lymph nodes
- ○ Omentum
- ○ Oral cavity
- ○ Ovary
- ○ Pancreas
- ○ Peritoneum
- ○ Pleura
- ○ Pleural effusion
- ○ Prostate
- ○ Rectum
- ○ Retroperitoneal mass
- ○ Skin
- ○ Small intestine
- ○ Spinal marrow
- ○ Spleen
- ○ Stomach
- ○ Testis
- ○ Uterus
- ○ Other

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

TUMOR EVALUATION [CHENDO] - CHENDO 1.0

Section Title: TUMOR EVALUATION
Instructions: Answer all required questions (marked with *)

1) Did patient perform tumor
evaluation? *   ○ Yes ○ No

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

Section Title: TARGET LESIONS

Instructions: Answer all required questions (marked with *)

1)
Has patient target lesions? *

○ Select…

○ Yes

○ No

○ Not applicable

Please complete table below only if patient performed tumor evaluation for target lesions

TARGET LESIONS

2) Location code	3) Description	4) Date of assessment	5) Method of measurement	6) Measurement of Target lesion
			○ Select… ○ CT-scan ○ MRI ○ PET ○ Physical Exam ○ Scintigraphy ○ Spiral CT ○ Ultrasonography ○ X-ray ○ Other	(mm)

○ Select...				
○ Adrenal				
○ Ascites				
○ Bone				
○ Bone marrow				
○ Breast				
○ Cervix				
○ CNS				
○ Colon				
○ Esophagus				
○ Head/neck				
○ Kidney				
○ Liver				
○ Lung				
○ Lymph nodes				
○ Omentum				
○ Oral cavity				
○ Ovary				
○ Pancreas				
○ Peritoneum				
○ Pleura				
○ Pleural effusion				
○ Prostate				
○ Rectum				
○ Retroperitoneal mass				
○ Skin				
○ Small intestine				
○ Spinal marrow				
○ Spleen				
○ Stomach				
○ Testis				
○ Uterus				
○ Other				

7) SUM   (mm)

Protocol ID: _____　　　　Study Subject ID:_____

Study Name: _____　　　　Interviewer Name:_____

Site: _____　　　　　　　　Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: NON TARGET LESIONS

Instructions: Answer all required questions (marked with *)

1)
Has patient non target lesions? *　　○ Select...　　　Please complete table below only if patient performed tumor evaluation for non target lesions

　　　　　　　　　　　　　　　　　○ Yes

　　　　　　　　　　　　　　　　　○ No

　　　　　　　　　　　　　　　　　○ Not applicable

NON TARGET LESIONS

2) Location code (site)	3) Description	4) Date of assessment	5) Method of measurement
			○ Select... ○ CT-scan ○ MRI ○ PET ○ Physical Exam ○ Scintigraphy ○ Spiral CT ○ Ultrasonography ○ X-ray ○ Other

- ○ Select...
- ○ Adrenal
- ○ Ascites
- ○ Bone
- ○ Bone marrow
- ○ Breast
- ○ Cervix
- ○ CNS
- ○ Colon
- ○ Esophagus
- ○ Head/neck
- ○ Kidney
- ○ Liver
- ○ Lung
- ○ Lymph nodes
- ○ Omentum
- ○ Oral cavity
- ○ Ovary
- ○ Pancreas
- ○ Peritoneum
- ○ Pleura
- ○ Pleural effusion
- ○ Prostate
- ○ Rectum
- ○ Retroperitoneal mass
- ○ Skin
- ○ Small intestine
- ○ Spinal marrow
- ○ Spleen
- ○ Stomach
- ○ Testis
- ○ Uterus
- ○ Other

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: RESPONSE

Instructions: Answer all required questions (marked with *)

1) Response Target lesions *
- ○ Select…
- ○ CR=Complete Response
- ○ PR=Partial Response
- ○ SD=Stable Disease
- ○ Not all evaluated
- ○ PD=Progressive Disease
- ○ NE=Inevaluable
- ○ Not applicable

2) Response Non Target lesions *
- ○ Select…
- ○ CR=Complete Response
- ○ Non-CR/non-PD
- ○ NE=Inevaluable
- ○ Non PD / or not all evaluated
- ○ PD=progressive disease
- ○ NL=New lesions
- ○ Not applicable

3) Overall Response *
- ○ Select…
- ○ CR=Complete Response
- ○ PR=Partial Response
- ○ SD=Stable Disease
- ○ NE=Inevaluable
- ○ PD=Progressive Disease
- ○ Not applicable

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

TUMOR MARKERS [CHENDO] - CHENDO 1.0

Section Title: TUMOR MARKERS
Instructions: Answer all required questions (marked with *)

1) Was tumor markers examinations performed? * ○ Yes ○ No

2) Date of tumor markers examinations *

3) CA 15-3 *
○ Select...
○ Normal
○ Abnormal NCS
○ Abnormal CS

4) CA 15-3 Value *   (KU/L)

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

URINE ANALYSIS [CHENDO] - CHENDO 1.0

Section Title: URINE ANALYSIS
Instructions: Answer all required questions (marked with *)

1) Was Urine analysis performed? * ○ Select...
○ Yes
○ No

2) Date of Urine analysis *

3) Hemoglobin *
○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

4) Hemoglobin value
○ Select...
○ Absent
○ Present

5) Proteins *
○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

6) Proteins value

7) Glucose *
○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

8) Glucose value

9) Bilirubin *
○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

10) Bilirubin value

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

ADVERSE EVENTS [CHENDO] - CHENDO 1.0

Section Title: ADVERSE EVENTS
Instructions: Answer all required questions (marked with *)

1) Has any adverse event been observed? *   ○ Yes ○ No

ADVERSE EVENTS											
2) Nr	**3) Adverse event**	**4) Specify (if requested)**	**5) GRADE**	**6) SAE**	**7) Start date**	**8) End Date**	**9) Relationship with study Treatment**	**10) If Yes specify treatment correlation**	**11) if related to CHT compound, specify**		**12) Outcome**
○ Select... ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10 ○ 11 ○ 12 ○ 13 ○ 14 ○ 15 ○ 16 ○ 17 ○ 18 ○ 19 ○ 20 ○ 21 ○ 22 ○ 23 ○ 24 ○ 25 ○ 26 ○ 27 ○ 28 ○ 29 ○ 30 ○ 31 ○ 32 ○ 33	○ Select... ○ Acne ○ Allergic/hypersensitivity reactions ○ Alteration of Lipid levels ○ Anemia ○ Anxiety/Confusion ○ Asthenia/Fatigue ○ Bone mineral density loss ○ Cardiac toxicity, specify ○ Cough ○ Diarrhea ○ Dyspnoea ○ Enhanced perspiration odor ○ Edema ○ Erythema/Rush ○ Febrile neutropenia ○ Fever ○ Hair loss ○ Headhache ○ Hot flashes ○ Hypercalcemia ○ Hypertension ○ Hypotension ○ Hyrsutism ○ Infection, specify ○ Liver toxicity, specify ○ Loss of appetite ○ Mood disturbance with nervousness and insomnia ○ Mucositis		○ Select... ○ Grade 0 ○ Grade 1 ○ Grade 2 ○ Grade 3 ○ Grade 4 ○ Grade 5	○ Select... ○ Yes ○ No			○ Select... ○ Not related ○ Not likely ○ Possible ○ Probable ○ Certain/Definite ○ Not Available	○ Select... ○ Exemestane ○ Letrozole ○ Anastrozole ○ CHT compound ○ CHT+HT ○ Unknown		○ Select... ○ Resolved ○ Resolved with sequelae ○ Unresolved ○ Unknown ○ Death

○ 34	○ Nausea	
○ 35	○ Nervous system disorders, specify	
○ 36	○ Neutropenia	
○ 37	○ Pain, specify	
○ 38	○ Oil skin	
○ 39	○ Polycythaemia	
○ 40	○ Pruritus	
○ 41	○ Renal toxicity, specify	
○ 42		
○ 43	○ Respiratory system toxicity, specify	
○ 44	○ Sexual disturbance	
○ 45	○ Skin toxicity, specify	
○ 46	○ Stypsis	
○ 47	○ Stomatitis	
○ 48	○ Sweating	
○ 49	○ Swelling	
○ 50	○ Tachycardia	
○ 51	○ Taste disturbance	
○ 52	○ Thrombocytopenia	
○ 53	○ Urinary incontinence	
○ 54	○ Voice alteration	
○ 55	○ Vomiting	
○ 56	○ Weight gain	
○ 57	○ Weight loss	
○ 58	○ Other, specify	
○ 59		
○ 60		
○ 61		
○ 62		
○ 63		
○ 64		
○ 65		
○ 66		
○ 67		
○ 68		
○ 69		
○ 70		
○ 71		
○ 72		
○ 73		
○ 74		
○ 75		
○ 76		
○ 77		
○ 78		
○ 79		
○ 80		
○ 81		
○ 82		
○ 83		
○ 84		
○ 85		
○ 86		

○ 87												
○ 88												
○ 89												
○ 90												
○ 91												
○ 92												
○ 93												
○ 94												
○ 95												
○ 96												
○ 97												
○ 98												
○ 99												
○ 100												

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

BIOMARKERS [CHENDO] - CHENDO 1.0

Section Title: BIOMARKERS
Instructions: Answer all required questions (marked with *)

1) Was blood sample for circulating
   biomarkers assessment collected
   (other than CTC)? *
   - ○ Select...
   - ○ Yes
   - ○ No

2) Timepoint (Circulating markers
   other than CTC) *
   - ○ Select...
   - ○ Screening
   - ○ Treatment phase
   - ○ EoT
   - ○ Unscheduled

3) Date of sample collection *

4) Was blood sample for CTCs
   assessment collected? *
   - ○ Select...
   - ○ Yes
   - ○ No

5) Timepoint CTC *
   - ○ Select...
   - ○ Screening
   - ○ 6-8 wks after treatment start
   - ○ 2-4 wks after last treatment
   - ○ Unscheduled

6) Date of sample collection *

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

CARDIOLOGIC ASSESSMENT [CHENDO] - CHENDO 1.0

Section Title: 12-lead ECG
Instructions: Answer all required questions (marked with *)

1) Was a ECG performed? *   ○ Yes   ○ No

2) Date of ECG *

3) ECG result *   ○ Select…

                       ○ Normal

                       ○ Abnormal, not clinically significant

                       ○ Abnormal, clinically significant

4) Please specify *

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

Section Title: Echocardiography or MUGA Scan
Instructions: Answer all required questions (marked with *)

1) Was cardiological evaluation performed? *

○ Yes ○ No

2) What kind of cardiological evaluation was performed? *

○ Select…

○ Echocardiography

○ MUGA Scan

3) Date of echocardiography *

4) Date of MUGA Scan *

5) Interpretation *

○ Select…

○ Normal

○ Abnormal

6) If abnormal, please specify *

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

COAGULATION TESTS [CHENDO] - CHENDO 1.0

Section Title: COAGULATION
Instructions: Answer all required questions (marked with *)

1) Were Coagulation test performed? ○ Yes ○ No
  *

2) Date of Coagulation tests *

3) PT (INR) *   ○ Select…   4) PT (INR) value
                       ○ Not done
                       ○ Normal
                       ○ Abnormal NCS
                       ○ Abnormal CS

5) aPTT (ratio) *   ○ Select…   6) aPTT (ratio) value
                       ○ Not done
                       ○ Normal
                       ○ Abnormal NCS
                       ○ Abnormal CS

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

COMORBIDITIES AND SYMPTOMS [CHENDO] - CHENDO 1.0

Section Title: COMORBIDITIES
Instructions: Answer all required questions (marked with *)

1)
Does patient have any
comorbidities? *
  ○
Yes   **Please specify any concomitant medication in the Concomitant Medication**
  ○ No   **section**

2) Cardiovascular pathology *
  ○ Select…
  ○ Yes
  ○ No

3) If yes, specify *

4) Date of diagnosis *

5) Concomitant therapy *
  ○ Select…
  ○ Yes
  ○ No
  ○ Unknown

6) Pulmonary pathology *
  ○ Select…
  ○ Yes
  ○ No

7) If yes, specify *

8) Date of diagnosis *

9) Concomitant therapy *
  ○ Select…
  ○ Yes
  ○ No
  ○ Unknown

10) Gastrointestinal/Hepatobiliary
pathology *
  ○ Select…
  ○ Yes
  ○ No

11) If yes, specify *

12) Date of diagnosis *

13) Concomitant therapy *
  ○ Select…
  ○ Yes
  ○ No
  ○ Unknown

14) Metabolic/ Endocrine pathology *
  ○ Select…
  ○ Yes
  ○ No

15) If yes, specify *

16) Date of diagnosis *

17) Concomitant therapy *
  ○ Select…
  ○ Yes
  ○ No
  ○ Unknown

18) Musculoskeletal pathology *
  ○ Select…
  ○ Yes
  ○ No

19) If yes, specify *

20) Date of diagnosis *

21) Concomitant therapy *

○ Select...
○ Yes
○ No
○ Unknown

22) Dermatologic pathology *   ○ Select...   23) If yes, specify *
                                     ○ Yes
                                     ○ No

24) Date of diagnosis *   25) Concomitant therapy *   ○ Select...
                                                                                             ○ Yes
                                                                                             ○ No
                                                                                             ○ Unknown

26) Reproductive pathology *   ○ Select...   27) If yes, specify *
                                     ○ Yes
                                     ○ No

28) Date of diagnosis *   29) Concomitant therapy *   ○ Select...
                                                                                             ○ Yes
                                                                                             ○ No
                                                                                             ○ Unknown

30) Renal/urinary tracts pathology * ○ Select...   31) If yes, specify *
                                      ○ Yes
                                      ○ No

32) Date of diagnosis *   33) Concomitant therapy *   ○ Select...
                                                                                             ○ Yes
                                                                                             ○ No
                                                                                             ○ Unknown

34) Allergy *   ○ Select...   35) If yes, specify *
                                      ○ Yes
                                      ○ No

36) Date of diagnosis *   37) Concomitant therapy *   ○ Select...
                                                                                             ○ Yes
                                                                                             ○ No
                                                                                             ○ Unknown

38) Neurologic/ Psichiatric pathology * ○ Select...   39) If yes, specify *
                                         ○ Yes
                                         ○ No

40) Date of diagnosis *   41) Concomitant therapy *   ○ Select...
                                                                                             ○ Yes
                                                                                             ○ No
                                                                                             ○ Unknown

42) Other *   ○ Select...   43) If yes, specify *
                                      ○ Yes
                                      ○ No

44) Date of diagnosis *   45) Concomitant therapy *

○ Select...
○ Yes
○ No
○ Unknown

46) Other *
○ Select...
○ Yes
○ No

47) If yes, specify *

48) Date of diagnosis *

49) Concomitant therapy *
○ Select...
○ Yes
○ No
○ Unknown

50) Other *
○ Select...
○ Yes
○ No

51) If yes, specify *

52) Date of diagnosis *

53) Concomitant therapy *
○ Select...
○ Yes
○ No
○ Unknown

Protocol ID: _____   Study Subject ID:_____
Study Name: _____   Interviewer Name:_____
Site: _____   Interview Date:_____
Event Name: _____
Event Date: _____

| **Section Title: SYMPTOMS** |
| Instructions: Answer all required questions (marked with *) |

1) Does patient have any symptoms? ○ Yes
   *   ○ No

2) Nausea *   ○ Select...
                           ○ Yes
                           ○ No

3) If yes, grade *   ○ Select...   4) Start date *
                           ○ 1
                           ○ 2
                           ○ 3
                           ○ 4

5) Vomiting *   ○ Select...
                           ○ Yes
                           ○ No

6) If yes, grade *   ○ Select...   7) Start date *
                           ○ 1
                           ○ 2
                           ○ 3
                           ○ 4

8) Constipation *   ○ Select...
                           ○ Yes
                           ○ No

9) If yes, grade *   ○ Select...   10) Start date *
                           ○ 1
                           ○ 2
                           ○ 3
                           ○ 4

11) Diarrhea *   ○ Select...
                           ○ Yes
                           ○ No

12) If yes, grade *   ○ Select...   13) Start date *
                           ○ 1
                           ○ 2
                           ○ 3
                           ○ 4

14) Stomatitis *   ○ Select...
                           ○ Yes
                           ○ No

15) If yes, grade *   ○ Select...   16) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

17) Fatigue *   ○ Select...
                            ○ Yes
                            ○ No

18) If yes, grade *   ○ Select...   19) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

20) Fever *   ○ Select...
                            ○ Yes
                            ○ No

21) If yes, grade *   ○ Select...   22) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

23) Skin *   ○ Select...   24) If yes, specify *
                            ○ Yes
                            ○ No

25) If yes, grade *   ○ Select...   26) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

27) Pain *   ○ Select...   28) If yes, specify *
                            ○ Yes
                            ○ No

29) If yes, grade *   ○ Select...   30) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

31) Respiratory system *   ○ Select...   32) If yes, specify *
                            ○ Yes
                            ○ No

33) If yes, grade *   ○ Select...   34) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

35) Neurologic system *   36) If yes, specify *

○ Select...
○ Yes
○ No

37) If yes, grade *   ○ Select...   38) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

39) Other *   ○ Select...   40) If yes, specify *
                            ○ Yes
                            ○ No

41) If yes, grade *   ○ Select...   42) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

43) Other *   ○ Select...   44) If yes, specify *
                            ○ Yes
                            ○ No

45) If yes, grade *   ○ Select...   46) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

47) Other *   ○ Select...   48) If yes, specify *
                            ○ Yes
                            ○ No

49) If yes, grade *   ○ Select...   50) Start date *
                            ○ 1
                            ○ 2
                            ○ 3
                            ○ 4

○ Select...
○ Yes
○ No

Protocol ID: _____ Study Subject ID:_____

Study Name: _____ Interviewer Name:_____

Site: _____ Interview Date:_____

Event Name: _____

Event Date: _____

CONCOMITANT MEDICATION [CHENDO] - CHENDO 1.0

Section Title: CONCOMITANT MEDICATION
Instructions: Answer all required questions (marked with *)

1) Does the patient receive any concomitant medication since study entry? *   ○ Select… ○ Yes ○ No

CONCOMITANT MEDICATION

2) Concomitant medication	3) Total daily dose	4) Unit	5) If "other", please specify	6) Route	7) If "other", please specify	8) Frequency	9) If "other", please specify	10) Start date	11) End date	12) Ongoing?	13) Indication	14) Please specify in case of AE, Concomitant Disease or Other
		○ Select… ○ capsule ○ drops ○ g ○ mg ○ ml ○ syringe ○ tablet ○ IU ○ vial ○ other		○ Select… ○ Oral ○ Intravenous ○ Subcutaneous ○ Intramuscolar ○ Topical ○ Other		○ Select… ○ Once daily ○ Twice a day ○ Three times a day ○ Every other day ○ weekly ○ Monthly ○ On demand ○ Other				☐ Ongoing	○ Select… ○ AE ○ As per protocol ○ Concomitant disease ○ Prophylaxis ○ Other	

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

DISEASE PROGRESSION [CHENDO] - CHENDO 1.0

Section Title: DISEASE PROGRESSION
Instructions: Answer all required questions (marked with *)

1) Did the patient have progression of disease? *
- ◯ Select…
- ◯ Yes
- ◯ No
- ◯ Unknown

2) Disease progression during: *
- ◯ Select…
- ◯ Chemotherapy
- ◯ Aromatase Inhibitors
- ◯ Chemotherapy AND Aromatase Inhibitors

3) Progressive disease (imaging-based)? *
- ◯ Select…
- ◯ Yes
- ◯ No

4) Date of Progressive disease (imaging-based) *

5) Clinical progressive disease? *
- ◯ Select…
- ◯ Yes
- ◯ No

6) Specify *

7) Date of Clinical progressive disease *

Protocol ID: _____ Study Subject ID:_____

Study Name: _____ Interviewer Name:_____

Site: _____ Interview Date:_____

Event Name: _____

Event Date: _____

ELIGIBILITY [CHENDO] - CHENDO 1.0

Section Title: INCLUSION CRITERIA
Instructions: Answer all required questions (marked with *)

1) Age ≥ 18 years * ◯ Yes ◯ No

2) Histological diagnosis of HER2-negative luminal breast cancer (ER>10% of tumor cells), determined by local laboratory on most recent available tumor tissue. * ◯ Yes ◯ No

3) Locally advanced (not susceptible to locoregional therapy) or metastatic disease (herein globally defined as "advanced breast cancer (ABC)"). * ◯ Yes ◯ No

4) Candidate to chemotherapy-based treatment per the investigator best judgment; e.g. because of disease aggressiveness, short disease-free interval, elevated Ki67 [if available on a metastatic site], low expression of hormone receptors, extended visceral involvement, visceral involvement at risk for organ failure, uncontrolled symptoms), according to Associazione Italiana di Oncologia Medica (AIOM) Guidelines (2016 edition). * ◯ Yes ◯ No

5) Postmenopausal women, or premenopausal women undergoing treatment with LHRH analog, or men (either receiving treatment with LHRH analog or not). * ◯ Yes ◯ No

6) Measurable disease according to RECIST 1.1 criteria, or not measurable but evaluable disease. * ◯ Yes ◯ No

◯ Yes ◯ No

7) No prior chemotherapy for advanced disease. Up to two prior lines of endocrine therapy for ABC, as well as targeted therapies (such as palbociclib and/or everolimus or investigative targeted therapies) administered as part of a prior hormonal regimen for ABC, are allowed. *

8) Eastern Cooperative Oncology Group performance status (ECOGPS) ≤2 (see Appendix A). *
   ○ Yes ○ No

9) Adequate organ (renal, hepatic, bone marrow, cardiac) functions. *
   ○ Yes ○ No

10) Female participants of child bearing potential and male participants whose partner is of child bearing potential must be willing to use effective contraception during the study period and for 4 months thereafter. Effective contraception methods include: total abstinence (when this is in line with the preferred and usual lifestyle of the subject); tubal ligation; male sterilization; combination of the placement of an intrauterine device or intrauterine system and barrier methods of contraception with spermicidal suppository. *
   ○ Yes ○ No

11) Participant is willing and able to give informed consent for participation in the study. *
   ○ Yes ○ No

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: EXCLUSION CRITERIA

Instructions: Answer all required questions (marked with *)

1) Any prior chemotherapy for advanced breast cancer * ⭘ Yes ⭘ No

2) Resistance to both non-steroidal and steroidal aromatase inhibitors, eg patients who progressed while on or within 12 months after the end of an aromatase inhibitor in the adjuvant setting and who progressed while on an aromatase inhibitor (of a different class) in the metastatic setting, or patients who progressed to both classes of aromatase inhibitors administered as two distinct lines of therapy for metastatic disease. * ⭘ Yes ⭘ No

3) Patients who have not recovered from adverse events due to prior therapies to grade ≤1 (excluding alopecia). * ⭘ Yes ⭘ No

4) Active central nervous system metastases. * ⭘ Yes ⭘ No

5) History of allergic reactions attributed to compounds of similar chemical or biologic composition to the chemotherapeutic or endocrine agents used in the study. * ⭘ Yes ⭘ No

6) Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (patients with history of hepatitis B must undergo prophylactic therapy with lamivudine or other agent according to infectious disease consultation), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. * ⭘ Yes ⭘ No

⭘ Yes ⭘ No

7) Prior history of non-breast malignancy (except for adequately controlled basal cell carcinoma of the skin, carcinoma in situ of the cervix, in situ carcinoma of the bladder), unless treated with curative intent and disease free for at least 3 years. *

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

Section Title: PATIENT INFORMATION
Instructions: Answer all required questions (marked with *)

1) ECOG performance status *   ○ 0 ○ 1 ○ 2 ○ 3

2) Ethnic origin *
- ○ Select…
- ○ Caucasian/White
- ○ Black
- ○ Asian
- ○ Other

3)
Menopausal state *
- ○ Select…
- ○ Post-menopausal
- ○ Premenopausal (undergoing treatment with LHRH analog)
- ○ Not applicable

4) Date of last menstural cycle (at least month and year) *

5) Date of informed consent signature *

6) Was FFPE tumor sample collected? ○ Yes ○ No *

7) Date of collection

Treatment choice

8) Type of chemotherapy regimen *
- ○ Select…
- ○ Anthracycline and taxane
- ○ Taxanes without anthracycline
- ○ Anthacycline without taxanes
- ○ Capecitabine or other fluoropyrimidines
- ○ Other

9) Type of Aromatase inhibitors *
- ○ Select…
- ○ Steroidal
- ○ Non-steroidal

10) Previous endocrine therapy for
ABC *

　○ Select...

　○ Pre-treated

　○ Not pre-treated

11) Notes

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

Section Title: PROCEED TO RANDOMIZATION
Instructions: Answer all required questions (marked with *)

1)
Do you want to proceed to Patient
Randomization? *

   ◯ Select...

   ◯ Yes

   ◯ No

Please verify that all Screening CRFs have been filled and save this CRF as complete.

2) Specify reason *

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

ELIGIBILITY CONFIRMATION [CHENDO] - CHENDO 1.0

Section Title: ELIGIBILITY CONFIRMATION
Instructions: Answer all required questions (marked with *)

Please ensure that you have filled all the Screening CRFs pages before proceeding to Randomization

1) Based on Inclusion/Exclusion Criteria and on screening assessments, is the patient eligible and can be randomized? *
○ Yes ○ No

2) Specify reason *
○ Select...
○ Inclusion Criteria
○ Exlcusion Criteria
○ Other

3) Inclusion Criteria N. *
○ Select...
○ 1
○ 2
○ 3
○ 4
○ 5
○ 6
○ 7
○ 8
○ 9

4) Exclusion criteria N. *
○ Select...
○ 1
○ 2
○ 3
○ 4
○ 5
○ 6
○ 7
○ 8
○ 9
○ 10

5) Other, specify *

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

END OF TREATMENT [CHENDO] - CHENDO 1.0

Section Title: REASON FOR END OF TREATMENT
Instructions: Answer all required questions (marked with *)

To be performed within 30 days of the last treatment administration

1) Last study treatment
   administration date *

2)
REASON FOR END OF TREATMENT
*

○ Select…

○ Treatment completed
according to protocol

○ Progression (imaging-based)

○ Pregnancy

○ Clinical progression

○ Unacceptable toxicity

○ Patient withdrew consent

○ Investigator's decision

○ Death

○ Protocol violation

○ Lost to follow-up

○ Other

Please complete End of Study
in case of death or lost
to follow-up

3) If Clinical progression, please
   specify *

4) If Unacceptable toxicity, please
   specify *

5) If Patient withdrew consent, please   (the date)
   specify the date *

6) If Investigator's decision, please
   specify *

7) If Protocol violation, please specify
   *

8) If Other, please specify *

Protocol ID: _____

Study Name: _____

Site: _____

Event Name: _____

Event Date: _____

Study Subject ID:_____

Interviewer Name:_____

Interview Date:_____

FOLLOW UP [CHENDO] - CHENDO 1.0

Section Title: FOLLOW UP
Instructions: Answer all required questions (marked with *)

To be performed every 3 months until Disease Progression

1) Did the patient perform Follow Up visit? * ○ Yes
　　　　　　　　　　　　　　　　　　　　　　 ○ No

2) Fup Date *

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

HORMONAL RECEPTORS ASSAY [CHENDO] - CHENDO 1.0

Section Title: HORMONAL RECEPTORS ASSAY
Instructions: Answer all required questions (marked with *)

1) Estrogen Receptors *
- ○ Select...
- ○ Positive
- ○ Negative
- ○ Not done

2) Estrogen Receptors value   (%)

3) Progesteron Receptors *
- ○ Select...
- ○ Positive
- ○ Negative
- ○ Not done

4) Progesteron Receptors value   (%)

5) HER2 evaluated? *
- ○ Yes
- ○ No

6) Method of evaluation *
- ☐ IHC
- ☐ FISH

7) IHC *
- ○ Select...
- ○ 1+
- ○ 2+
- ○ 3+

8) FISH *
- ○ Select...
- ○ Positive
- ○ Negative

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____   Interview Date:_____

Event Name: _____

Event Date: _____

LABORATORY EXAMINATION [CHENDO] - CHENDO 1.0

Section Title: HEMATHOLOGY
Instructions: Answer all required questions (marked with *)

1) Was Hematological examination performed? *   ○ Yes ○ No

2) Date of Hematological examination *

3) WBC *
- ○ Select...
- ○ Not done
- ○ Normal
- ○ Abnormal NCS
- ○ Abnormal CS

4) WBC value   (x10^9/L)

5) Hgb *
- ○ Select...
- ○ Not done
- ○ Normal
- ○ Abnormal NCS
- ○ Abnormal CS

6) Hgb value   (g/dl)

7) PLT *
- ○ Select...
- ○ Not done
- ○ Normal
- ○ Abnormal NCS
- ○ Abnormal CS

8) PLT value   (x10^9/L)

9) Neutrophils *
- ○ Select...
- ○ Not done
- ○ Normal
- ○ Abnormal NCS
- ○ Abnormal CS

10) Neutrophils value   (x10^9/L)

11) Lymphocytes *
- ○ Select...
- ○ Not done
- ○ Normal
- ○ Abnormal NCS
- ○ Abnormal CS

12) Lymphocytes value   (x10^9/L)

13) Monocyte *
- ○ Select...
- ○ Not done
- ○ Normal
- ○ Abnormal NCS
- ○ Abnormal CS

14) Monocyte value   (x10^9/L)

15) Eosinophils *

16) Eosinophils value   (x10^9/L)

○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

17) Basophils *

○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

18) Basophils value   (x10^9/L)

○ Select...
○ Not done
○ Normal
○ Abnormal NCS
○ Abnormal CS

Protocol ID: _____   Study Subject ID:_____

Study Name: _____   Interviewer Name:_____

Site: _____

Event Name: _____   Interview Date:_____

Event Date: _____

Section Title: SERUM CHEMISTRY

Instructions: Answer all required questions (marked with *)

---

1) Was Serum Chemistry examination ○ Yes ○ No
   performed? *

2) Date of Serum Chemistry
   examination *

3) Creatinine *   ○ Select...   4) Creatinine value   (mg/dl)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

5) Sodium *   ○ Select...   6) Sodium value   (mmol/L)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

7) Potassium *   ○ Select...   8) Potassium value   (mmol/L)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

9) Chloride *   ○ Select...   10) Chloride value   (mmol/L)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

11) Calcium *   ○ Select...   12) Calcium value   (mg/dl)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

13) Tot. bilirubin *   ○ Select...   14) Tot. bilirubin value   (mg/dl)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

15) Albumin *   ○ Select...   16) Albumin value   (g/L)
                         ○ Not done
                         ○ Normal
                         ○ Abnormal NCS
                         ○ Abnormal CS

17) ALAT/SGPT *   ○ Select...   18) ALAT/SGPT value   (U/L)
                               ○ Not done
                               ○ Normal
                               ○ Abnormal NCS
                               ○ Abnormal CS

19) Alk. Phos. *   ○ Select...   20) Alk. Phos. value   (U/L)
                               ○ Not done
                               ○ Normal
                               ○ Abnormal NCS
                               ○ Abnormal CS

21) Tot. protein *   ○ Select...   22) Tot. protein value   (g/L)
                               ○ Not done
                               ○ Normal
                               ○ Abnormal NCS
                               ○ Abnormal CS

○ Select...

○ Not done

○ Normal

○ Abnormal NCS

○ Abnormal CS

Appendix 6

Randomization List protocol final version

ID	Strato	Descrizione Strato	Descrizione Randomizzazione
1	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
2	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
3	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
4	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
5	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
6	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
7	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
8	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
9	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
10	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
11	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
12	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
13	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
14	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
15	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
16	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
17	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
18	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
19	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
20	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
21	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
22	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
23	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
24	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
25	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
26	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
27	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
28	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
29	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
30	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
31	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
32	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
33	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
34	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
35	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
36	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
37	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
38	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
39	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
40	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
41	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
42	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
43	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
44	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
45	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
46	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
47	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
48	IRCCS IRST	IRCCS IRST	ARM A: CT + AI

49	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
50	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
51	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
52	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
53	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
54	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
55	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
56	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
57	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
58	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
59	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
60	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
61	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
62	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
63	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
64	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
65	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
66	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
67	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
68	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
69	IRCCS IRST	IRCCS IRST	ARM A: CT + AI
70	IRCCS IRST	IRCCS IRST	ARM B: CT --> AI
71	Ravenna	Ravenna	ARM A: CT + AI
72	Ravenna	Ravenna	ARM B: CT --> AI
73	Ravenna	Ravenna	ARM A: CT + AI
74	Ravenna	Ravenna	ARM B: CT --> AI
75	Ravenna	Ravenna	ARM A: CT + AI
76	Ravenna	Ravenna	ARM B: CT --> AI
77	Ravenna	Ravenna	ARM B: CT --> AI
78	Ravenna	Ravenna	ARM A: CT + AI
79	Ravenna	Ravenna	ARM A: CT + AI
80	Ravenna	Ravenna	ARM B: CT --> AI
81	Ravenna	Ravenna	ARM B: CT --> AI
82	Ravenna	Ravenna	ARM A: CT + AI
83	Ravenna	Ravenna	ARM A: CT + AI
84	Ravenna	Ravenna	ARM B: CT --> AI
85	Ravenna	Ravenna	ARM A: CT + AI
86	Ravenna	Ravenna	ARM B: CT --> AI
87	Ravenna	Ravenna	ARM A: CT + AI
88	Ravenna	Ravenna	ARM B: CT --> AI
89	Ravenna	Ravenna	ARM B: CT --> AI
90	Ravenna	Ravenna	ARM A: CT + AI
91	Ravenna	Ravenna	ARM A: CT + AI
92	Ravenna	Ravenna	ARM B: CT --> AI
93	Ravenna	Ravenna	ARM B: CT --> AI
94	Ravenna	Ravenna	ARM A: CT + AI
95	Ravenna	Ravenna	ARM B: CT --> AI
96	Ravenna	Ravenna	ARM A: CT + AI
97	Ravenna	Ravenna	ARM A: CT + AI
98	Ravenna	Ravenna	ARM B: CT --> AI

99 Ravenna	Ravenna	ARM B: CT --> AI
100 Ravenna	Ravenna	ARM A: CT + AI
101 Ravenna	Ravenna	ARM A: CT + AI
102 Ravenna	Ravenna	ARM B: CT --> AI
103 Ravenna	Ravenna	ARM B: CT --> AI
104 Ravenna	Ravenna	ARM A: CT + AI
105 Ravenna	Ravenna	ARM A: CT + AI
106 Ravenna	Ravenna	ARM A: CT + AI
107 Ravenna	Ravenna	ARM B: CT --> AI
108 Ravenna	Ravenna	ARM B: CT --> AI
109 Ravenna	Ravenna	ARM B: CT --> AI
110 Ravenna	Ravenna	ARM A: CT + AI
111 Rimini	Rimini	ARM B: CT --> AI
112 Rimini	Rimini	ARM A: CT + AI
113 Rimini	Rimini	ARM B: CT --> AI
114 Rimini	Rimini	ARM A: CT + AI
115 Rimini	Rimini	ARM B: CT --> AI
116 Rimini	Rimini	ARM A: CT + AI
117 Rimini	Rimini	ARM A: CT + AI
118 Rimini	Rimini	ARM A: CT + AI
119 Rimini	Rimini	ARM B: CT --> AI
120 Rimini	Rimini	ARM B: CT --> AI
121 Rimini	Rimini	ARM A: CT + AI
122 Rimini	Rimini	ARM B: CT --> AI
123 Rimini	Rimini	ARM B: CT --> AI
124 Rimini	Rimini	ARM A: CT + AI
125 Rimini	Rimini	ARM A: CT + AI
126 Rimini	Rimini	ARM B: CT --> AI
127 Rimini	Rimini	ARM A: CT + AI
128 Rimini	Rimini	ARM B: CT --> AI
129 Rimini	Rimini	ARM A: CT + AI
130 Rimini	Rimini	ARM B: CT --> AI
131 Rimini	Rimini	ARM B: CT --> AI
132 Rimini	Rimini	ARM A: CT + AI
133 Rimini	Rimini	ARM A: CT + AI
134 Rimini	Rimini	ARM B: CT --> AI
135 Rimini	Rimini	ARM B: CT --> AI
136 Rimini	Rimini	ARM A: CT + AI
137 Rimini	Rimini	ARM A: CT + AI
138 Rimini	Rimini	ARM B: CT --> AI
139 Rimini	Rimini	ARM A: CT + AI
140 Rimini	Rimini	ARM B: CT --> AI
141 Rimini	Rimini	ARM B: CT --> AI
142 Rimini	Rimini	ARM A: CT + AI
143 Rimini	Rimini	ARM B: CT --> AI
144 Rimini	Rimini	ARM A: CT + AI
145 Rimini	Rimini	ARM B: CT --> AI
146 Rimini	Rimini	ARM A: CT + AI
147 Rimini	Rimini	ARM A: CT + AI
148 Rimini	Rimini	ARM B: CT --> AI

149 Rimini	Rimini	ARM B: CT --> AI
150 Rimini	Rimini	ARM A: CT + AI
151 Carpi	Carpi	ARM B: CT --> AI
152 Carpi	Carpi	ARM B: CT --> AI
153 Carpi	Carpi	ARM A: CT + AI
154 Carpi	Carpi	ARM A: CT + AI
155 Carpi	Carpi	ARM A: CT + AI
156 Carpi	Carpi	ARM B: CT --> AI
157 Carpi	Carpi	ARM A: CT + AI
158 Carpi	Carpi	ARM B: CT --> AI
159 Carpi	Carpi	ARM A: CT + AI
160 Carpi	Carpi	ARM B: CT --> AI
161 Carpi	Carpi	ARM B: CT --> AI
162 Carpi	Carpi	ARM A: CT + AI
163 Carpi	Carpi	ARM B: CT --> AI
164 Carpi	Carpi	ARM A: CT + AI
165 Carpi	Carpi	ARM A: CT + AI
166 Carpi	Carpi	ARM B: CT --> AI
167 Carpi	Carpi	ARM B: CT --> AI
168 Carpi	Carpi	ARM A: CT + AI
169 Carpi	Carpi	ARM B: CT --> AI
170 Carpi	Carpi	ARM A: CT + AI
171 Carpi	Carpi	ARM B: CT --> AI
172 Carpi	Carpi	ARM A: CT + AI
173 Carpi	Carpi	ARM A: CT + AI
174 Carpi	Carpi	ARM B: CT --> AI
175 Carpi	Carpi	ARM B: CT --> AI
176 Carpi	Carpi	ARM A: CT + AI
177 Carpi	Carpi	ARM A: CT + AI
178 Carpi	Carpi	ARM B: CT --> AI
179 Carpi	Carpi	ARM B: CT --> AI
180 Carpi	Carpi	ARM A: CT + AI
181 Carpi	Carpi	ARM A: CT + AI
182 Carpi	Carpi	ARM B: CT --> AI
183 Carpi	Carpi	ARM A: CT + AI
184 Carpi	Carpi	ARM B: CT --> AI
185 Carpi	Carpi	ARM A: CT + AI
186 Carpi	Carpi	ARM A: CT + AI
187 Carpi	Carpi	ARM B: CT --> AI
188 Carpi	Carpi	ARM B: CT --> AI
189 Carpi	Carpi	ARM B: CT --> AI
190 Carpi	Carpi	ARM A: CT + AI
191 Bologna	Bologna	ARM B: CT --> AI
192 Bologna	Bologna	ARM A: CT + AI
193 Bologna	Bologna	ARM B: CT --> AI
194 Bologna	Bologna	ARM A: CT + AI
195 Bologna	Bologna	ARM A: CT + AI
196 Bologna	Bologna	ARM B: CT --> AI
197 Bologna	Bologna	ARM B: CT --> AI
198 Bologna	Bologna	ARM A: CT + AI

199 Bologna	Bologna	ARM A: CT + AI
200 Bologna	Bologna	ARM B: CT --> AI
201 Bologna	Bologna	ARM A: CT + AI
202 Bologna	Bologna	ARM B: CT --> AI
203 Bologna	Bologna	ARM B: CT --> AI
204 Bologna	Bologna	ARM A: CT + AI
205 Bologna	Bologna	ARM B: CT --> AI
206 Bologna	Bologna	ARM A: CT + AI
207 Bologna	Bologna	ARM A: CT + AI
208 Bologna	Bologna	ARM B: CT --> AI
209 Bologna	Bologna	ARM A: CT + AI
210 Bologna	Bologna	ARM B: CT --> AI
211 Bologna	Bologna	ARM B: CT --> AI
212 Bologna	Bologna	ARM A: CT + AI
213 Bologna	Bologna	ARM B: CT --> AI
214 Bologna	Bologna	ARM A: CT + AI
215 Bologna	Bologna	ARM A: CT + AI
216 Bologna	Bologna	ARM B: CT --> AI
217 Bologna	Bologna	ARM B: CT --> AI
218 Bologna	Bologna	ARM A: CT + AI
219 Bologna	Bologna	ARM B: CT --> AI
220 Bologna	Bologna	ARM A: CT + AI
221 Bologna	Bologna	ARM A: CT + AI
222 Bologna	Bologna	ARM B: CT --> AI
223 Bologna	Bologna	ARM A: CT + AI
224 Bologna	Bologna	ARM B: CT --> AI
225 Bologna	Bologna	ARM A: CT + AI
226 Bologna	Bologna	ARM B: CT --> AI
227 Bologna	Bologna	ARM A: CT + AI
228 Bologna	Bologna	ARM B: CT --> AI
229 Bologna	Bologna	ARM B: CT --> AI
230 Bologna	Bologna	ARM A: CT + AI
231 Piacenza	Piacenza	ARM B: CT --> AI
232 Piacenza	Piacenza	ARM A: CT + AI
233 Piacenza	Piacenza	ARM A: CT + AI
234 Piacenza	Piacenza	ARM B: CT --> AI
235 Piacenza	Piacenza	ARM B: CT --> AI
236 Piacenza	Piacenza	ARM A: CT + AI
237 Piacenza	Piacenza	ARM A: CT + AI
238 Piacenza	Piacenza	ARM A: CT + AI
239 Piacenza	Piacenza	ARM B: CT --> AI
240 Piacenza	Piacenza	ARM B: CT --> AI
241 Piacenza	Piacenza	ARM A: CT + AI
242 Piacenza	Piacenza	ARM B: CT --> AI
243 Piacenza	Piacenza	ARM A: CT + AI
244 Piacenza	Piacenza	ARM B: CT --> AI
245 Piacenza	Piacenza	ARM A: CT + AI
246 Piacenza	Piacenza	ARM B: CT --> AI
247 Piacenza	Piacenza	ARM B: CT --> AI
248 Piacenza	Piacenza	ARM A: CT + AI

249 Piacenza	Piacenza	ARM A: CT + AI
250 Piacenza	Piacenza	ARM B: CT --> AI
251 Piacenza	Piacenza	ARM B: CT --> AI
252 Piacenza	Piacenza	ARM A: CT + AI
253 Piacenza	Piacenza	ARM A: CT + AI
254 Piacenza	Piacenza	ARM B: CT --> AI
255 Piacenza	Piacenza	ARM A: CT + AI
256 Piacenza	Piacenza	ARM B: CT --> AI
257 Piacenza	Piacenza	ARM B: CT --> AI
258 Piacenza	Piacenza	ARM A: CT + AI
259 Piacenza	Piacenza	ARM A: CT + AI
260 Piacenza	Piacenza	ARM B: CT --> AI
261 Piacenza	Piacenza	ARM B: CT --> AI
262 Piacenza	Piacenza	ARM A: CT + AI
263 Piacenza	Piacenza	ARM A: CT + AI
264 Piacenza	Piacenza	ARM B: CT --> AI
265 Piacenza	Piacenza	ARM B: CT --> AI
266 Piacenza	Piacenza	ARM A: CT + AI
267 Piacenza	Piacenza	ARM B: CT --> AI
268 Piacenza	Piacenza	ARM A: CT + AI
269 Piacenza	Piacenza	ARM B: CT --> AI
270 Piacenza	Piacenza	ARM A: CT + AI
271 Bari	Bari	ARM B: CT --> AI
272 Bari	Bari	ARM A: CT + AI
273 Bari	Bari	ARM B: CT --> AI
274 Bari	Bari	ARM A: CT + AI
275 Bari	Bari	ARM A: CT + AI
276 Bari	Bari	ARM B: CT --> AI
277 Bari	Bari	ARM B: CT --> AI
278 Bari	Bari	ARM A: CT + AI
279 Bari	Bari	ARM A: CT + AI
280 Bari	Bari	ARM B: CT --> AI
281 Bari	Bari	ARM B: CT --> AI
282 Bari	Bari	ARM A: CT + AI
283 Bari	Bari	ARM A: CT + AI
284 Bari	Bari	ARM B: CT --> AI
285 Bari	Bari	ARM B: CT --> AI
286 Bari	Bari	ARM A: CT + AI
287 Bari	Bari	ARM A: CT + AI
288 Bari	Bari	ARM B: CT --> AI
289 Bari	Bari	ARM B: CT --> AI
290 Bari	Bari	ARM A: CT + AI
291 Bari	Bari	ARM B: CT --> AI
292 Bari	Bari	ARM A: CT + AI
293 Bari	Bari	ARM A: CT + AI
294 Bari	Bari	ARM B: CT --> AI
295 Bari	Bari	ARM A: CT + AI
296 Bari	Bari	ARM B: CT --> AI
297 Bari	Bari	ARM A: CT + AI
298 Bari	Bari	ARM B: CT --> AI

299 Bari	Bari	ARM A: CT + AI
300 Bari	Bari	ARM B: CT --> AI
301 Bari	Bari	ARM B: CT --> AI
302 Bari	Bari	ARM A: CT + AI
303 Bari	Bari	ARM A: CT + AI
304 Bari	Bari	ARM B: CT --> AI
305 Bari	Bari	ARM A: CT + AI
306 Bari	Bari	ARM B: CT --> AI
307 Bari	Bari	ARM B: CT --> AI
308 Bari	Bari	ARM A: CT + AI
309 Bari	Bari	ARM A: CT + AI
310 Bari	Bari	ARM B: CT --> AI
311 Aviano	Aviano	ARM A: CT + AI
312 Aviano	Aviano	ARM B: CT --> AI
313 Aviano	Aviano	ARM B: CT --> AI
314 Aviano	Aviano	ARM A: CT + AI
315 Aviano	Aviano	ARM A: CT + AI
316 Aviano	Aviano	ARM B: CT --> AI
317 Aviano	Aviano	ARM A: CT + AI
318 Aviano	Aviano	ARM B: CT --> AI
319 Aviano	Aviano	ARM B: CT --> AI
320 Aviano	Aviano	ARM B: CT --> AI
321 Aviano	Aviano	ARM A: CT + AI
322 Aviano	Aviano	ARM A: CT + AI
323 Aviano	Aviano	ARM B: CT --> AI
324 Aviano	Aviano	ARM A: CT + AI
325 Aviano	Aviano	ARM A: CT + AI
326 Aviano	Aviano	ARM B: CT --> AI
327 Aviano	Aviano	ARM B: CT --> AI
328 Aviano	Aviano	ARM B: CT --> AI
329 Aviano	Aviano	ARM A: CT + AI
330 Aviano	Aviano	ARM A: CT + AI
331 Aviano	Aviano	ARM A: CT + AI
332 Aviano	Aviano	ARM B: CT --> AI
333 Aviano	Aviano	ARM B: CT --> AI
334 Aviano	Aviano	ARM A: CT + AI
335 Aviano	Aviano	ARM B: CT --> AI
336 Aviano	Aviano	ARM A: CT + AI
337 Aviano	Aviano	ARM A: CT + AI
338 Aviano	Aviano	ARM B: CT --> AI
339 Aviano	Aviano	ARM A: CT + AI
340 Aviano	Aviano	ARM B: CT --> AI
341 Aviano	Aviano	ARM A: CT + AI
342 Aviano	Aviano	ARM B: CT --> AI
343 Aviano	Aviano	ARM B: CT --> AI
344 Aviano	Aviano	ARM A: CT + AI
345 Aviano	Aviano	ARM A: CT + AI
346 Aviano	Aviano	ARM B: CT --> AI
347 Aviano	Aviano	ARM B: CT --> AI
348 Aviano	Aviano	ARM A: CT + AI

349 Aviano	Aviano	ARM A: CT + AI
350 Aviano	Aviano	ARM B: CT --> AI
351 Parma	Parma	ARM B: CT --> AI
352 Parma	Parma	ARM A: CT + AI
353 Parma	Parma	ARM A: CT + AI
354 Parma	Parma	ARM B: CT --> AI
355 Parma	Parma	ARM A: CT + AI
356 Parma	Parma	ARM B: CT --> AI
357 Parma	Parma	ARM A: CT + AI
358 Parma	Parma	ARM B: CT --> AI
359 Parma	Parma	ARM B: CT --> AI
360 Parma	Parma	ARM A: CT + AI
361 Parma	Parma	ARM A: CT + AI
362 Parma	Parma	ARM B: CT --> AI
363 Parma	Parma	ARM B: CT --> AI
364 Parma	Parma	ARM A: CT + AI
365 Parma	Parma	ARM B: CT --> AI
366 Parma	Parma	ARM A: CT + AI
367 Parma	Parma	ARM A: CT + AI
368 Parma	Parma	ARM B: CT --> AI
369 Parma	Parma	ARM B: CT --> AI
370 Parma	Parma	ARM A: CT + AI
371 Parma	Parma	ARM A: CT + AI
372 Parma	Parma	ARM B: CT --> AI
373 Parma	Parma	ARM B: CT --> AI
374 Parma	Parma	ARM A: CT + AI
375 Parma	Parma	ARM B: CT --> AI
376 Parma	Parma	ARM A: CT + AI
377 Parma	Parma	ARM A: CT + AI
378 Parma	Parma	ARM B: CT --> AI
379 Parma	Parma	ARM A: CT + AI
380 Parma	Parma	ARM B: CT --> AI
381 Parma	Parma	ARM B: CT --> AI
382 Parma	Parma	ARM A: CT + AI
383 Parma	Parma	ARM A: CT + AI
384 Parma	Parma	ARM B: CT --> AI
385 Parma	Parma	ARM B: CT --> AI
386 Parma	Parma	ARM A: CT + AI
387 Parma	Parma	ARM A: CT + AI
388 Parma	Parma	ARM B: CT --> AI
389 Parma	Parma	ARM B: CT --> AI
390 Parma	Parma	ARM A: CT + AI
391 Genova	Genova	ARM B: CT --> AI
392 Genova	Genova	ARM A: CT + AI
393 Genova	Genova	ARM B: CT --> AI
394 Genova	Genova	ARM A: CT + AI
395 Genova	Genova	ARM B: CT --> AI
396 Genova	Genova	ARM A: CT + AI
397 Genova	Genova	ARM A: CT + AI
398 Genova	Genova	ARM B: CT --> AI

399 Genova	Genova	ARM A: CT + AI
400 Genova	Genova	ARM B: CT --> AI
401 Genova	Genova	ARM A: CT + AI
402 Genova	Genova	ARM B: CT --> AI
403 Genova	Genova	ARM B: CT --> AI
404 Genova	Genova	ARM A: CT + AI
405 Genova	Genova	ARM B: CT --> AI
406 Genova	Genova	ARM A: CT + AI
407 Genova	Genova	ARM B: CT --> AI
408 Genova	Genova	ARM A: CT + AI
409 Genova	Genova	ARM B: CT --> AI
410 Genova	Genova	ARM A: CT + AI
411 Genova	Genova	ARM B: CT --> AI
412 Genova	Genova	ARM A: CT + AI
413 Genova	Genova	ARM B: CT --> AI
414 Genova	Genova	ARM A: CT + AI
415 Genova	Genova	ARM A: CT + AI
416 Genova	Genova	ARM B: CT --> AI
417 Genova	Genova	ARM B: CT --> AI
418 Genova	Genova	ARM B: CT --> AI
419 Genova	Genova	ARM A: CT + AI
420 Genova	Genova	ARM A: CT + AI
421 Genova	Genova	ARM A: CT + AI
422 Genova	Genova	ARM B: CT --> AI
423 Genova	Genova	ARM B: CT --> AI
424 Genova	Genova	ARM A: CT + AI
425 Genova	Genova	ARM A: CT + AI
426 Genova	Genova	ARM B: CT --> AI
427 Genova	Genova	ARM B: CT --> AI
428 Genova	Genova	ARM A: CT + AI
429 Genova	Genova	ARM A: CT + AI
430 Genova	Genova	ARM B: CT --> AI
431 Cremona	Cremona	ARM A: CT + AI
432 Cremona	Cremona	ARM B: CT --> AI
433 Cremona	Cremona	ARM A: CT + AI
434 Cremona	Cremona	ARM B: CT --> AI
435 Cremona	Cremona	ARM B: CT --> AI
436 Cremona	Cremona	ARM A: CT + AI
437 Cremona	Cremona	ARM B: CT --> AI
438 Cremona	Cremona	ARM A: CT + AI
439 Cremona	Cremona	ARM A: CT + AI
440 Cremona	Cremona	ARM B: CT --> AI
441 Cremona	Cremona	ARM A: CT + AI
442 Cremona	Cremona	ARM B: CT --> AI
443 Cremona	Cremona	ARM A: CT + AI
444 Cremona	Cremona	ARM B: CT --> AI
445 Cremona	Cremona	ARM A: CT + AI
446 Cremona	Cremona	ARM A: CT + AI
447 Cremona	Cremona	ARM B: CT --> AI
448 Cremona	Cremona	ARM B: CT --> AI

449 Cremona	Cremona	ARM B: CT --> AI
450 Cremona	Cremona	ARM A: CT + AI
451 Cremona	Cremona	ARM A: CT + AI
452 Cremona	Cremona	ARM B: CT --> AI
453 Cremona	Cremona	ARM B: CT --> AI
454 Cremona	Cremona	ARM A: CT + AI
455 Cremona	Cremona	ARM A: CT + AI
456 Cremona	Cremona	ARM B: CT --> AI
457 Cremona	Cremona	ARM B: CT --> AI
458 Cremona	Cremona	ARM A: CT + AI
459 Cremona	Cremona	ARM A: CT + AI
460 Cremona	Cremona	ARM B: CT --> AI
461 Cremona	Cremona	ARM B: CT --> AI
462 Cremona	Cremona	ARM A: CT + AI
463 Cremona	Cremona	ARM A: CT + AI
464 Cremona	Cremona	ARM B: CT --> AI
465 Cremona	Cremona	ARM A: CT + AI
466 Cremona	Cremona	ARM B: CT --> AI
467 Cremona	Cremona	ARM A: CT + AI
468 Cremona	Cremona	ARM B: CT --> AI
469 Cremona	Cremona	ARM B: CT --> AI
470 Cremona	Cremona	ARM A: CT + AI

Randomization List protocol Amendment 1.0

ID	Strato	Descrizione Strato	Descrizione Randomizzazione
471	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
472	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
473	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
474	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
475	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
476	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
477	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
478	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
479	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
480	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
481	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
482	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
483	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
484	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
485	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
486	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
487	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
488	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
489	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
490	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
491	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
492	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
493	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
494	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI

495 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
496 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
497 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
498 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
499 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
500 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
501 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
502 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
503 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
504 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
505 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
506 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
507 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
508 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
509 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
510 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
511 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
512 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
513 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
514 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
515 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
516 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
517 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
518 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
519 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
520 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
521 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
522 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
523 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
524 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
525 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
526 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
527 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
528 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
529 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
530 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
531 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
532 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
533 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
534 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
535 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
536 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
537 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
538 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
539 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
540 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
541 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
542 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
543 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
544 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI

545 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
546 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
547 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
548 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
549 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
550 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
551 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
552 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
553 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
554 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
555 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
556 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
557 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
558 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
559 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
560 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
561 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
562 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
563 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
564 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
565 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
566 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
567 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
568 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
569 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
570 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
571 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
572 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
573 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
574 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
575 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
576 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
577 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
578 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
579 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
580 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
581 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
582 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
583 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
584 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
585 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
586 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
587 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
588 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
589 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
590 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
591 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
592 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
593 S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
594 S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI

595	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
596	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
597	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
598	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
599	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
600	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
601	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
602	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
603	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
604	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
605	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
606	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
607	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
608	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
609	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
610	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
611	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
612	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
613	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
614	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
615	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
616	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
617	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
618	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI
619	S_CHENDO	STUDIO CHENDO	ARM B: CT --> AI
620	S_CHENDO	STUDIO CHENDO	ARM A: CT + AI