Official Title: **OBSERVATIONAL AND PROSPECTIVE STUDY TO DEVELOP PREDICTIVE AND PROGNOSTIC TOOLS FOR OPTIMIZING THERAPY WITH BEVACIZUMAB FRONTLINE CANCER THERAPY IN PATIENTS WITH METASTATIC HER 2-NEGATIVE AND AGGRESSIVE DISEASE CRITERIA. ARGO STUDY. 16th DECEMBER 2016**

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STATISTICAL ANALYSIS PLAN FINAL For NON-INTERVENTIONAL STUDIES

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1. BACKGROUND AND STUDY OBJECTIVES

1.1 RATIONALE AND BACKGROUND

Bevacizumab is a humanized antibody directed against VEGF-A, a potent angiogenic agent shown to be a determinant in the growth and spread of cancer in humans. The use of the combination of different cytotoxic agents with bevacizumab in advanced breast cancer has shown consistent benefits in Progression Free Survival (PFS) and response rates. However, no trial or meta-analysis has identified a benefit in overall survival for the bevacizumab combinations. The absence of global survival before the IMELDA study data may have limited the incorporation of this active agent in clinical practice.

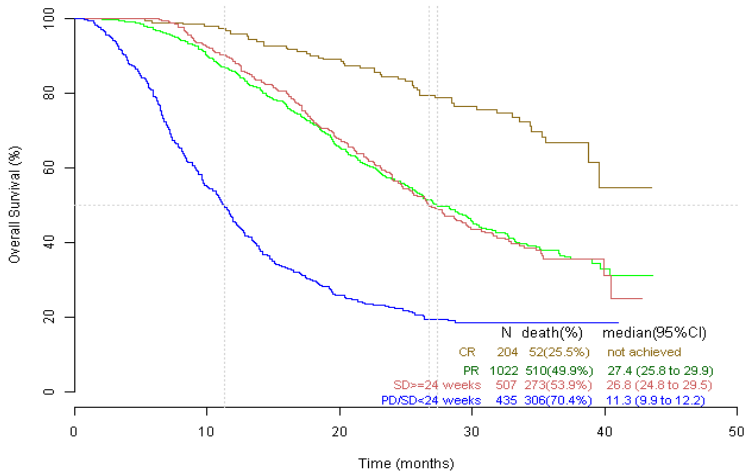
The IMELDA study (Gligorov et al. 2014), one of the second-generation clinical trials on breast cancer with bevacizumab as first line treatment, has explored an original argument. All MBC patients received docetaxel plus bevacizumab as first line therapy (winning arm of the AVADO (Miles DW, et al. 2010) study) for a total of six cycles [in the absence of progression]). Patients underwent a response evaluation after the sixth cycle; and those that did not present progression criteria (stable disease or response) were randomized to maintenance with bevacizumab or to bevacizumab in combination with a second cytostatic (capecitabine), until disease progression or toxicity. The study shows a dramatic benefit in PFS (PFS: 4.3 vs. 11.9 months; Hazard ratio 0.38; p <.0001) and Overall Survival (OS) (median OS 23.7 vs 39.0 months; Hazard ratio 0.43; p= 0.0003) in favor of the combination. This study optimizes the efficacy of bevacizumab, confirming its synergic effect with chemotherapy. Therefore, prolonging low intensity chemotherapy with capecitabine plus bevacizumab in sensitive patients achieves survival benefits similar to those reported with trastuzumab in the HER2-positive population.

The main limitation of this study is that fails in making an early detection of the patients who do not benefit from the treatment with bevacizumab. Since the absence of efficacy was defined as progression within the first six cycles of chemotherapy, it did not limit the administration of bevacizumab to truly sensitive patients. The IMELDA study stresses the need for an early detection of tumor resistance to the combination.

These data globally concur with an observation made by another clinical study ATHENA (unpublished and blinded data). This study included over 2.200 patients treated first line with a bevacizumab-containing regimen, and it showed that overall survival maintained a close correlation with the efficacy of the first line treatment. Patients with a CR (n = 204; Median OS not achieved, but >40 months), partial objective response (n = 1.022; OS 27.4 months) or SD for more than 24 weeks (n = 507, OS 26.8 months) obtained higher survival figures than the patients who displayed progression before 24 weeks (n = 435, OS 11.3 months). In total, 20% patients did not reach clinical benefit criteria (progression within 24 weeks) to the first line treatment (figure 1).

Interestingly, when we compare the OS figures from this study and IMELDA, the median OS for the control "sensitive" arm (maintaining bevacizumab after six CT cycles) moves to 27.2 months (23.7 and median treatment duration on the initial phase is 3.5 months); similar to the 27.8 months from the non-resistant group (SD >24 weeks + PR + CR; n = 1,733)

Figure 1. Correlation between overall survival according to best response to bevacizumab first line treatment. Total sample analysis (N=2264)



*96 patients are excluded. Their responses were not evaluated in the study.

** The median survival was not estimated because the survival probability in CR group is higher than 50%.

CR: Complete response, PR: Partial response, SD: Stable Disease, PD: Progressive disease, 95%CI: 95% confidence interval.

The determination of Circulating Tumor Cells (CTCs) in blood of Metastatic Breast Cancer (MBC) patients is a well-established prognostic and predictive marker of efficacy/resistance. An initial determination of CTCs superior to 5 CTCs/ 7.5 ml confers a worse PFS and OS rates to first line chemotherapy compared to lower CTC values indicating that it works both as an early marker for treatment resistance and survival. Additionally, the persistence of values ≥ 5 CTCs/ 7.5 ml after the first cycle/weeks of chemotherapy is an early marker for resistance (Cristofanilli M et al. 2004).

Recently, the SWOG S0500 study (Smerage JB et al. 2014) study has explored the ability of CTCs as prognostic and predictor markers to lead to early treatment changes in patients with resistant criteria tumors. The study confirmed the possibility of monitoring CTCs to predictive early sensitivity to the first line treatment as well as prognosis. However, it failed in the primary study objective of the study, as no difference in PFS or OS was observed when patients who maintained ≥ 5 CTCs after 3-5 weeks on therapy

were randomized to either maintain the first line therapy (n = 62; PFS/OS 3.5 and 10.7 months) or changed to a non-cross-resistant regimen (n = 60; 4.6 and 12.5 months).

However, the SWOG S0500 study allowed for the expected patterns of population with basal CTCs < 5 and ≥ 5 to be established, as well as those of the populations with normalization / no-normalization on the 3-5 weeks determination.	% Patients	PFS	OS
Basal CTCs <5	45-49%	11,1	35
Basal CTCs ≥ 5 and subsequent <5	29-32%	8.9	23
Basal and subsequent ≥ 5CTCs	22%	4.9	13

Due to the aforementioned, our hypothesis supports that an early CTC monitoring; basal and after second cycle in patients initiating first line paclitaxel plus bevacizumab chemotherapy; will identify two populations with a very different PFS results; the population with maintained high CTC levels (≥ 5 CTCs in both determinations) will progress shortly expecting a median PFS of 4.0 months, while those with a low CTC determination (basal or after the second cycle) will achieve median PFS in the range of 13 months.

The CTC monitoring could become a useful tool for early prediction of sensitivity/resistance to bevacizumab regimens. The SWOG S0500 trial has confirmed that there is no benefit from changing the cytotoxic agent or combination for patients with a resistant CTC profile to the first regimen. Seen from a different point of view, it makes sense that for patients with no CTC normalization following the first cycles there is little marginal benefit from maintaining bevacizumab instead of moving the patient to single agent paclitaxel.

This study will identify a powerful and easy predictive/prognostic marker to drive patients under bevacizumab.

1.2 STUDY OBJECTIVES

Effectiveness Objectives

To determine the predictive capacity of early CTCs monitoring predefined as sensitive (<5 CTCs after cycle 2) and resistant (≥5 CTCs after cycle 2) in terms of Clinical Benefit Rate (defined as patients achieving partial or complete response as well as tumor stabilization ≥ 24 weeks) for patients with HER2-negative aggressive metastatic breast cancer treated with standard first line chemotherapy with paclitaxel-bevacizumab.

The secondary effectiveness objectives of this study are as follows:

- To evaluate the validity of early CTCs monitoring, as predictor of overall response (OR) progression free survival (PFS) and overall survival (OS) and toxicity grading (3-4) (by Common Terminology Criteria for Adverse Events [CTCAE] v4 criteria)
- To estimate the optimal cut-off of CTCs in sample data for CBR, OR, PFS and to compare good and poor prognostic groups in PFS and treatment response.
- To correlate CEA and CA15.3 with CTC levels

Safety Objectives

The safety objectives for this study are as follows:

- To evaluate the incidence of adverse events, serious adverse events, events of special interest and events that lead to treatment discontinuation in two prognostic groups and the total sample.
- To evaluate the validity of <5 CTCs/ 7.5 mL after second chemotherapy cycle as predictive factor of toxicity grading (3-4) (by CTCAE v4 criteria)
- To estimate the optimal cut-off of CTCs in sample data for toxicity grading (3-4)

2. STUDY DESIGN

2.1 DESCRIPTION OF STUDY DESIGN

This is a multicenter, observational, prospective study in patients with metastatic breast cancer who initiate standard treatment with bevacizumab in combination with paclitaxel. The development of the study involves the prospective collection of data for 18 months after initiation of therapy. The decision of treatment is independent of the inclusion and in no case shall be subject by the inclusion of the patient in the study. The visits coincide with the patient perform regularly to monitor your condition, without interfering in any way with the clinical practice of the researcher.

To ensure the observational nature of it, these data should always be collected and when available in the medical record or may be obtained during the interview with the same in such visits do not apply any diagnostic or therapeutic intervention.

For the primary objective two CTCs determinations will be performed as follows:

1. Determination of basal CTCs determination (within the 21 days prior to day 1 /cycle 1 treatment) will be done in eligible patients after the sign of the informed consent

2. A second determination of CTCs in the 7 days prior to day 1/cycle 3 (before any treatment administration of cycle 3)

Paclitaxel and bevacizumab will be administered as per clinical protocol in each institution. The expected bevacizumab dose will be 10 mg/kg day 1 and 15 every four weeks. It is recommended to maintain bevacizumab until confirmation of tumor progression or unacceptable toxicity. All patients will be followed for up to 18 months from the start of treatment (including those who discontinue study treatment for any reason other than PD).

2.2 DATA SOURCES

The investigator will use the electronic case report form (eCRF) with the assigned patient number to enter medically relevant data collected from medical charts during the study visits. Medical records will also be used to capture additional information.

2.3 PRIMARY OUTCOME MEASURES

The primary effectiveness variable in this study is as follows:

- The primary variable is the clinical benefit rate during the study follow-up which will be assessed according to CTC levels. Clinical benefit rate is defined as patients achieving partial or complete response as well as tumor stabilization ≥ 24 weeks. It will be compared clinical benefit rates between CTC sensitive group (<5 CTCs after cycle 2) and resistance group (≥5 CTCs after cycle 2).

2.4 SECONDARY OUTCOME MEASURES

2.4.1 Secundary Effectiveness Variables

The effectiveness variables of this study are as follows:

- Levels of CTC
- Medical history.
- Treatments for metastatic breast cancer
- Concomitant medication.
- Variables related with treatment response
- Biomarkers (CEA and CA 15.3)
- Progression free survival and OS

2.4.2 Safety Variables

Collection of adverse events, serious adverse events, events of special interest, events which lead to treatment or study discontinuation and laboratory parameters.

2.5 OTHER OUTCOME MEASURES

- Clinical and pathological variables: age, pre/post menopausical status, tumor status, histology, hormone receptor and HER2 status, concomitant medications, ECOG functional status, weight, height, chemotherapy (doses and scheme), radiotherapy or surgery to treat cancer disease and medical history, and toxicities that may occur during the study period.

- CTC determinations.

- Laboratory assessment (per usual clinical practice) usually contains:

    - Haematology: haemoglobin, hematocrit, platelet count, red blood cell (RBC) count, white blood cell (WBC) count, absolute differential count (neutrophils, eosinophils, lymphocytes, monocytes, basophils, other cells).

    - Serum chemistry: sodium, potassium, chloride, blood glucose, blood urea nitrogen (BUN) or urea, creatinine, calcium, total bilirubin, total protein or albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH).

    - Coagulation: International Normalized Ratio (INR) and activated partial thromboplastin time (aPTT).

    - Urinalysis: by dipstick test.

    - Pregnancy test.

- Time variables as for example: date of first breast cancer diagnosis, date of metastatic disease, start date of the first line treatment, date of progression and date of death (if happens during the study)

2.6 DETERMINATION OF SAMPLE SIZE

The calculation of sample size was based on the determination of the main objective: " To determine the predictive capacity of early CTCs monitoring predefined as sensitive (<5 CTCs after cycle 2) and resistant (>5 CTCs after cycle 2) in terms of Clinical Benefit Rate (defined as patients achieving partial or complete response as well as tumor stabilization ≥ 24 weeks) for patients with HER2-negative aggressive metastatic breast cancer treated with standard first line chemotherapy with paclitaxel-bevacizumab". The precision values, power, etc., of the other secondary objectives are achieved, accordingly, depending on the size determined by the primary endpoint.

The primary endpoint is the clinical benefit rate at follow-up study. The investigator's hypothesis is that CBR will be 35% and 70% for CTC-resistance and sensitive groups, respectively. We assume the ratio between CTC-Resistance and CTC sensitive group will be 1:4. We accept an alpha risk of 0.05 two-sided and a beta risk of 0.2. We anticipate a drop-out rate of 10%. We will analyze the data with a Chi-square test for two independent proportions.

Finally, 22 subjects in CTC-Resistance group and 88 subjects in CTC-sensitive group are estimated. Therefore a total of 110 patients are needed. Sample size has been estimated with the GRANMO V7.12 April 2012 program.

3. STATISTICAL METHODS

Upon completion of the study, after recording all data up to and including the last patient, the database shall be closed and the statistical analysis performed.

The statistical analysis methods proposed below constitute a summary of the methods to be used on the data collected in order to fulfil the study objectives.

A general description shall be given of the variables included in the study. Distributions of absolute and relative frequencies of the qualitative variables shall be presented, along with measures of central tendency and dispersion (mean, standard deviation, median, minimum and maximum) for the quantitative variables.

Where an inferential analysis is required, parametric tests shall be used for continuous variables and nonparametric tests for ordinal, categorical and nonparametric variables. The hypothesis tests used shall be two-tailed in all cases, with a significance level of 0.05. For variables that do not follow a normal (or parametric) distribution, Mann Witney (for unpaired data) or Wilcoxon (for paired data) hypothesis tests shall be used. For the analysis of the contingency tables and comparison of proportions and/or frequency distributions, the chi-squared test (or Fisher's exact test where appropriate) shall be used.

It will be calculated the Pearson correlation coefficient (or Spearman correlation coefficient) to evaluate the association between two continuous variables.

Studies of ROC curves will be carried out looking for cut points that maximize sensitivity and specificity. It will be calculated the area under the curve (AUC) with 95% confidence interval.

The "time to event" variables (i.e. progression-free survival, overall survival, etc.) will be analyzed using the Kaplan-Meier method. The survival curves will be compared with the Log-Rank test method.

Absences of data shall not be accounted for and shall be considered missing data.

95% confidence intervals shall be given for the principal quantitative variables for results associated with the primary objective and the principal secondary variables.

The Statistical Package for the Social Sciences (SPSS) software shall be used to carry out the analysis.

3.1 ANALYSIS POPULATIONS

Effectiveness analyses will be based on all enrolled patients who meet selection criteria. Safety analyses will include all patients who received at least one dose of paclitaxel or bevacizumab (Safety Population).

Descriptive analyses of CRF will be based on Safety Population.

Information referring to the selection criteria is recorded in the CRF as a binary variable (Yes/No) for each written item. Five of these criteria may be verified with the data collected in the CRF. The criteria that may be verified are defined below:

1- Patients aged ⩾ 18 years.

The age will be calculated on the date of informed consent.

2- Patients with HER2-negative MBC. Mandatory to have the HER2/estrogen receptor(ER)/progesterone receptor (PR) status.

Patients must have the data indicated (HER2, Re and PR) and HER2 must be negative.

3- ECOG 0-2.

Patients must have the data indicated and this must be 0-2.

4- Signature of informed consent.

Patients must have information on the form "Informed consent" and the data of the date of signature must be before the date of basal visit.

The patients must indicate "Yes" to the question "Has the patient signed the informed consent?" The date of the signature should also be indicated and it must be before the date of basal visit.

5- Pregnant or breastfeeding patient. A serum pregnancy test will be taken up to 7 days prior to study treatment, or up to 14 days prior to study treatment (in the latter case another urine test will be taken within 7 days prior to study treatment to confirm the result). Exclusion criterion.

Patients must have information on the form "Pregnancy test" and the result must be negative.

3.2 EFFECTIVENESS ANALYSIS

CTC groups: there are going to be determined patient groups based on early CTCs monitoring (sensitive patients, resistant patients). **This definition of CTC groups will be used in all the analyses proposed below except in cases where another CTC group is indicated.**

- Sensitive patients: <5 CTCs after cycle 2.

- Resistant patients: ≥5 CTCs after cycle 2.

The categorical variable to be described (N, %) is:

- CTC group (Sensitive, Resistant).

3.2.1 Survival

3.2.1.1 Follow-up time

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variable will be shown:

- Follow-up time, defined as time from start of treatment to date of last available follow-up at the time of data cut-off.

3.2.1.2 Exitus

Categorical variables to be described:

- Exitus (Yes/No).

- The number of exitus.

- Reason of exitus (death by AE and the rest of death will be considered like cause PD).

3.2.1.3 Progression free survival (PFS)

PFS is defined as the time, in months, from the start of treatment to disease progression, or death from any cause, whichever occurs first.

The median PFS will be described (Kaplan-Meier Analysis) as well as the number of events and censored cases (Kaplan-Meier Analysis).

To detect patients with progression it will be considered data collected in the treatment with Bevacizumab period and without discontinuations. It will be assigned the first date of progression detected.

If patient has not progressed but has died it will be assigned the date of Exitus detected in "Study completion/Early termination".

If patient has not progressed and has not died during treatment and has not a first subsequent treatment with Bevacizumab he will be censored at the time of starting the first subsequent treatment (it will be considered a case of discontinuation).

If patient has not progressed and has not died during the treatment with Bevacizumab (without discontinuations) will be censored on the date of the last available follow-up.

3.2.1.4 Overall survival (OS)

It is defined as the time, in months, from the start of treatment to until death from any cause.

If patient dies the date of Exitus registered in the "Study completion/Early termination" form will be used.

Patients who have not died will be censored on the date of the last available follow-up.

- The median OS will be described as well as the number of events and censored cases (Kaplan-Meier Analysis).

3.2.2 Primary Effectiveness Analysis

3.2.2.1 Primary Effectiveness Analysis. Definitions and descriptive analyses

- Best treatment response: it will be calculated the best treatment response (information collected in the treatment period visits). In patients without subsequent treatments and with Study completion/Early termination visit the treatment response (in this visit) will be considerate too.

- Depending on the best treatment response, the overall response rate (OR= Complete Response + Partial Response) will be calculated and described, as well as the 95% confidence interval.

The categorical variable to be described (N, %, 95% confidence interval) is:

- OR (Yes/No).

- Depending on the best treatment response, the clinical benefit rate (CBR=Complete Response + Partial Response + Tumor stabilization) will be calculated and described, as well as the 95% confidence interval.

The categorical variable to be described (N, %, 95% confidence interval) is:

- CBR (Yes/No).

3.2.2.2 Primary Effectiveness Analysis. Comparative analyses

- It will be compared CBR (Yes/No) between CTC groups.

3.2.3 Secondary Effectiveness Analysis. Validity of early CTCs monitoring as predictor of OR, PFS and OS

To evaluate the validity of early CTCs monitoring, as predictor of overall response (OR) progression free survival (PFS) and overall survival (OS) and toxicity grading (3-4) (by Common Terminology Criteria for Adverse Events [CTCAE] v4 criteria). The following comparative analyses will be performed:

- It will be compared OR (Yes/No) between CTC groups.
- It will be compared PFS between CTC groups.
- It will be compared OS between CTC groups.
- It will be compared Toxicity grading 3-4 (Yes/No) between CTC groups (See in 3.3.5 and 3.3.6., in Safety Analyses).

3.2.4 Secondary Effectiveness Analysis. Optimal cut-off of CTCs for OR, CBR and PFS and compare good and poor prognosis groups in treatment response and PFS

3.2.4.1 Optimal cut-off of CTCs for OR

To estimate the optimal cut-off of CTCs in sample data for OR it will get the ROC curve using the continuous variable CTCs after cycle 2.

The classification groups will be defined by OR (No/Yes).

- It will be calculated the area under the curve (AUC) with 95% confidence interval.
- It will be calculated the optimal cut-off, that maximize Sensitivity and Specificity.

To compare good and poor prognostic groups in PFS and treatment response the following comparative analyses will be performed:

- It will be compared PFS between the new CTC groups obtained with ROC curve.
- It will be compared OR between the new CTC groups obtained with ROC curve.

3.2.4.2 Optimal cut-off of CTCs for CBR

To estimate the optimal cut-off of CTCs in sample data for CBR it will get the ROC curve using the continuous variable CTCs after cycle 2.

The classification groups will be defined by CBR (No/Yes).

- It will be calculated the area under the curve (AUC) with 95% confidence interval.
- It will be calculated the optimal cut-off, that maximize Sensitivity and Specificity.

To compare good and poor prognostic groups in PFS and treatment response the following comparative analyses will be performed:

- It will be compared PFS between the new CTC groups obtained with ROC curve.
- It will be compared OR between the new CTC groups obtained with ROC curve.

3.2.4.3 Optimal cut-off of CTCs for PFS

To estimate the optimal cut-off of CTCs in sample data for PFS it will get the ROC curve using the continuous variable CTCs after cycle 2.

The classification groups will be defined by PFS (disease progression, or death from any cause, whichever occurs first, see definition in 3.2.1.3).

- It will be calculated the area under the curve (AUC) with 95% confidence interval.
- It will be calculated the optimal cut-off, that maximize Sensitivity and Specificity.

To compare good and poor prognostic groups in PFS and treatment response the following comparative analyses will be performed:

- It will be compared PFS between the new CTC groups obtained with ROC curve.
- It will be compared OR between the new CTC groups obtained with ROC curve.

3.2.5 Secondary Effectiveness Analysis. Correlation between CTC levels and Biomarkers

To correlate CEA and CA15.3 with CTC levels the following analyzes will be made:

- It will be calculated the Pearson correlation coefficient (or Spearman correlation coefficient) to evaluate the association between CTC level with and CEA.
- It will be calculated the Pearson correlation coefficient (or Spearman correlation coefficient) to evaluate the association between CTC level with and CA 15.3.

These analyses will carried out in basal visit and in the treatment visit in which the CTC levels are determined.

3.3 SAFETY ANALYSES

Adverse events will be coded with the MedDRA dictionary.

3.3.1 Toxicity

All adverse events related to the study medication will be considered as toxicities, i.e., all events specified as “Related” to bevacizumab or paclitaxel.

Toxicity analysis will be provided per patient. To obtain toxicity per patient, the maximum grade will be calculated for each of the toxicities recorded over all the treatment cycles of each patient and the following will be described:

- Number of patients with at least one toxicity.
- Number of patients with different toxicities reported according to grade (GI, GII, GIII, GIV and GV).

3.3.1.1 Toxicity. CTC groups analyses

- It will be compared Toxicity (Yes/No) between CTC groups.
- It will be described in each CTC group: number of patients with different toxicities reported according to grade (GI, GII, GIII, GIV and GV).

3.3.2 Adverse events

This analysis will include all adverse events occurring in patients throughout the study. To obtain adverse events per patient, the maximum grade will be calculated for each of the adverse events recorded over all the treatment cycles of each patient and the following will be described:

- Number of patients with at least one adverse event.
- Number of patients with different adverse events reported according to grade (GI, GII, GIII, GIV and GV).

3.3.2.1 Adverse events. CTC groups analyses

- It will be compared Adverse event (Yes/No) between CTC groups.
- It will be described in each CTC group: number of patients with different adverse events reported according to grade (GI, GII, GIII, GIV and GV).

3.3.3 Serious adverse events. Events of special interest

The categorical variables to be described (N, %) are:

- Patients with at least one serious adverse event.
- Patients with at least one events of special interest.

In addition, a list of serious adverse event and events of special interest occurring in the patients along with their characteristics will be provided.

3.3.3.1 Serious adverse events. CTC groups analyses

- It will be compared Serious adverse event (Yes/No) between CTC groups.

In addition, a list of serious adverse event occurring in the patients along with their characteristics will be provided. In this list it will specified the CTD group too.

3.3.3.2 Events of special interest. CTC groups analyses

- It will be compared Event of special interest (Yes/No) between CTC groups.

In addition, a list of events of special interest occurring in the patients along with their characteristics will be provided. In this list it will specified the CTD group too.

3.3.4 Adverse events that lead to treatment discontinuation

Adverse events lead to treatment discontinuation must be indicated in the Study completion/Early termination visit. The following will be described:

- Number of patients with at least one adverse events that lead to treatment discontinuation.
- A list with the adverse events specified.

3.3.4.1 Adverse events that lead to treatment discontinuation. CTC groups analyses

- It will be compared Adverse events that lead to treatment discontinuation (Yes/No) between CTC groups.

3.3.5 Validity of early CTCs monitoring as predictor of Toxicity grading 3-4

- It will be compared Toxicity grading 3-4 (Yes/No) between CTC groups.

3.3.6 Analysis. Optimal cut-off of CTCs for Toxicity grading 3-4

To estimate the optimal cut-off of CTCs in sample data for Toxicity grading 3-4 (Yes/No) it will get the ROC curve using the continuous variable CTCs after cycle 2.

The classification groups will be defined by Toxicity grading 3-4(Yes/No).

- It will be calculated the area under the curve (AUC) with 95% confidence interval.
- It will be calculated the optimal cut-off, that maximize Sensitivity and Specificity.

3.4 SENSITIVITY ANALYSES

Sensitivity analysis is not applicable for this study.

3.5 OTHER ANALYSES

3.5.1 Baseline descriptive analysis

Recruitment period

The inclusion dates of the first and last patient will be provided (date of signature of informed consent).

Graph of recruited patients

A list of participating sites and the investigators who participated at each site will be provided, indicating the number and percentage of patients recruited.

The number of patients recruited in each site will be represented by a bar chart.

Sociodemographic and anthropometric data

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variables will be shown:

- Age in years: Calculated on the day of signature of informed consent (date of informed consent - date of birth).
- Weight (kg).
- Height (cm).
- BMI: Calculate as weight/height$(m)^2$.

The categorical variables to be described (N, %) are:

- Sex.
- Race.

Medical history: relevant past or active diseases

The number and percentage of patients who have indicated each of the diseases specified in the CRF will be described.

For patients with any of the diseases, it will be indicated whether this disease continues or not.

Phyical exploration

It will be described (N, %) the status (Normal, Abnormal, ND) of each of the physical exploration systems.

Medical history related to cancer

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variable will be shown:

- Time from first tumor diagnosis to study inclusion: Calculated in years on the day of signature of informed consent (date of informed consent – date of first tumor diagnosis).

The categorical variables to be described (N, %) are

- Age at diagnosis.
- Family history.
- Initial metastasis.
- Initial stage.
- Menopausal status.
- Initial stage.

Neoadjuvant treatment

The number and percentage of patients receiving neoadjuvant treatment and the different treatment regimens received will be described.

Surgical resection

The number and percentage of patients who underwent surgery will be described and the following characteristics:

- Mastectomy/Conservative surgery.
- Unilateral/Bilateral.
- Selective sentinel node biopsy.
- Lymph node emptying, and if so, description of:
    - Level.
    - Type of nodes.

- TNM staging.

Adjuvant treatment

The number and percentage of patients receiving adjuvant treatment will be described and the following characteristics:

- The number of patients who received radiotherapy.
- The number of patients with adjuvant chemotherapy and the different treatment regimens received.
- The number of patients with adjuvant hormonal therapy and the different types of treatment received.

Neoadjuvant/Adjuvant treatment

The number and percentage of patients receiving neoadjuvant and/or adjuvant treatment will be described and the following characteristics:

- The number of patients with neoadjuvant and/or adjuvant chemotherapy and the different treatment regimens received.

Locoregional recurrence

The number and percentage of patients experiencing locoregional recurrence will be described and the following characteristics:

- The number of patients with Mastectomy.
- The number of patients with chemotherapy and the different treatment regimens received.
- The number of patients with adjuvant hormonal therapy and the different types of treatment received.

Metastatic disease

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variables will be shown:

- Time from diagnosis of metastatic disease to study inclusion: Calculated in years on the day of signature of informed consent (date of informed consent - date of diagnosis of metastasis).
- Number of metastatic lesions at inclusion.

The categorical variables to be described (N, %) are:

- Histologic type.
- Histologic grade.
- Extracapsular extension.
- Biopsy of metastases.
- Histologic subtype.
- Location of metastases at inclusion.

Receptor status

- Receptor status: Estrogens.
- Receptor status: Progesterone.
- Receptor status: HER2.
- New variable will be created: Triple Negative:
    - Yes: Estrogens (-) and Progesterone (-) and HER2 (-)
    - No: [Estrogens (+) or Progesterone (+)] and HER2 (-)

Treatment for metastatic disease

The number of patients receiving hormonal therapy will be described and the different types of treatment received.

Disease status (ECOG)

The categorical variables to be described (N, %) are:

- ECOG

Laboratory data: CTCs

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variable will be shown:

- Baseline CTC levels

CTC level data will be provided in an Excel file by the sponsor.

Haematology:

The categorical variables to be described (N, %) are:

- Out of range (the analysis will be performed for each parameter in eCRF).
- Clinically significant (the analysis will be performed for each parameter in eCRF).

Serum chemistry:

The categorical variables to be described (N, %) are:

- Out of range (the analysis will be performed for each parameter in eCRF).
- Clinically significant (the analysis will be performed for each parameter in eCRF).

Coagulation:

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variable will be shown:

- INR.
- aPTT.

Urianalysis:

The categorical variables to be described (N, %) are:

- Proteins.

Pregnancy test:

The categorical variables to be described (N, %) are:

- Patients having serum determination.
- Patients having urine determination.
- Result of test for serum determination.

- Result of test for urine determination.

Biomarkers status: CEA / CA 15.3

The categorical variables to be described (N, %) are:

- Patients having CEA determination.
- Patients having CA 15.3 determination.

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variables will be shown:

- CEA value
- CA 15.3 value

3.5.2 Treatment period descriptive analysis

Laboratory data: CTCs

Descriptive statistics (mean, SD, median, Q1, Q3, minimum, maximum) of the following continuous variable will be shown:

- Treatment period CTC levels.

CTC level data will be provided in an Excel file by the sponsor.

3.5.3 Final visit or premature withdrawal

Reason for end of treatment

The following variables will be described (N,%):

- Scheduled study completion (24 months) / Premature withdrawal.
- Reason for premature withdrawal. In the case of adverse event it will be specified.

3.5.4 Concomitant medication

The following variables will be described (N,%):

- Active substante.

3.5.5 Additional analysis

- The number and percentage of patients in the next groups based in CTC levels will be obtained:
    - <5 Baseline CTC level and <5 CTCs after cycle 2
    - ≥5 Baseline CTC level and <5 CTCs after cycle 2
- It will be studied whether there are statistically significant differences between the triple negative patients (yes or no) and the initial CTC levels.
- It will be calculated the Pearson correlation coefficient (or Spearman correlation coefficient) to evaluate the association between initial CTC level with Number of metastatic lesions at inclusion.
- It will be studied if there is a relation between OR (CR+PR) and triple negative patients (Yes / No)
- The progression-free survival (PFS) between the groups defined by the response to treatment will be studied –OR (CR+PR)- (Kaplan-Meier Analysis). Long Rank test will be apliqued
- It will be studied if there is a relation between CTC (Sensitive / Resistance) and triple negative patients (Yes / No)
- It will be studied if there is a relation between CTC (decrease: second measure – first measure) and triple negative patients (Yes / No)
- Number of patients with Imelda scheme: For the treatments registered in the follow-up visit form, in the post-treatment period, the drugs collected in Treatment will be selected, sending the promoter the list with the name of the drug and the start date. In addition, in order to identify the scheme after Chemo + Avastin, for each patient, the end date of paclitaxel and bevacizumab will be included in the list (last end date recorded in the paclitaxel and bevacizumab treatment form, respectively). In this list the promoter will classify the treatments in the following groups:
    - Hormone + Avastin
    - or Xeloda + Avastin
    - or Avastin only

  In this way, the number and percentage of patients with Imelda scheme after the study treatment will be obtained.

3.6 HANDLING OF MISSING DATA

No handling of missing data will be done.

3.7 LIMITATIONS OF THE RESEARCH METHOD

Apart from those belonging to the design of the observational studies, no further potential limitations are detected.

4. REFERENCES

1. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. The New England journal of medicine 2004;351:781-791

2. Cristofanilli M, Hayes DF, Budd GT, et al: Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005, 23:1420-1430.A published erratum appears in J Clin Oncol 2005, 23:4808

3. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC.

4. Giuliano M, Giordano A, Jackson S, et al. Circulating tumor cells as early predictivos of metastatic spread in breast cancer patients with limited metastatic dissemination. BCR 2014;16:440

5. Gligorov J, Doval D, Bines J, et al. Maintenance capecitabine and bevacizumab versus bevacizumab alone after initial first-line bevacizumab and docetaxel for patients with HER2-negative metastatic breast cancer (IMELDA): a randomised, open-label, phase 3 trial. The Lancet Oncology 2014;

6. Jemal, A. et al. Cancer statistics, 2007. CA Cancer J Clin 57, 43-66 (2007).

7. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:3239-3247

8. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology: Breast cancer v.2.2008. http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf. (2008).

9. Smerage JB, Barlow WE, Hortobagyi GN, et al. Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 2014.

10. Parkin,D.M., Bray,F., Ferlay,J. et al. Global cancer statistics, 2002. CA Cancer J. Clin. 55, 74-108 (2005).

Appendix 1
Data Collection Overview for PDC (as per Standard of Care)

Data Collection (available data will be collected; no additional diagnostic or monitoring procedures shall be applied to the patients outside of routine clinical practice)	Basal (the screening and the first observation visit can coincide)	Data Collected during Treatment Period (every 9-12 weeks)	Data Collected at Study Completion/ Early Termination Visit	Data Collected during Follow-Up (every 9-12 weeks)
Selection criteria	x			
Informed consent [a]	x			
Demographic data	x	x[b]	x[b]	x[b]
Vital signs[c]	x	x	x	x
Medical history[d]	x			
Physical exploration[e]	x	x	x	x
Concomitant medications[f]	x	x	x	x
Cancer related data	x	x	x	x
CTC determination[g]	x	x		
Haematology	x	x	x	x
Serum chemistry	x	x	x	x
Coagulation	x			
Urinalysis	x	x	x	x
Pregnancy test[h]	x	x	x	
Biomarkers status[i]	x			
Treatment efficacy evaluation		x	x	x
Adverse events		x	x	x

[a] Written informed consent must be obtained before any data collection.
[b] Only weight.
[c] Include measurements of systolic and diastolic blood pressure while the patient is in a seated position.
[d] Medical history includes clinically significant diseases surgeries, smoking history, use of alcohol and drugs of abuse, any abnormality. Any relevant change in the medical history during the follow up will be considered as an adverse event.

[e] Include an evaluation of the head, eye, ear, nose, and throat, and the cardiovascular, dermatological, musculoskeletal, respiratory, GI, genitourinary, and neurological systems. Any relevant change during the follow up will be considered as an adverse event.
[f] Include all medications (e.g. prescription medicines, over-the-counter medicines, herbal/homeopathic remedies, nutritional supplements) used by the patient; and changes from the initial in the follow up visits.
[g] CTC determinations: within the 21 days prior to day 1/ cycle 1 treatment and in the 7 days prior to day 1/cycle 3 (before any treatment administration of cycle 3).
[h] Only in premenopausical women
[i] Included CEA and CA 15.3.