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**

NCT Number: NCT02613208

Document Date: Protocol Version 2: 01-February-2017

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**
PROTOCOL NUMBER:	ML29756 /ROC-BEV-2015-01
VERSION NUMBER:	2
DATE OF LAST VERSION:	**16TH December 2016**
MEDICINAL PRODUCT{S}:	Paclitaxel-Bevacizumab
SPONSOR:	Roche Farma S.A.
DATE FINAL:	**16TH December 2016**

PROTOCOL FINALIZATION SIGNATURE PAGE

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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DATE FINAL:	16TH December 2016

This protocol was finalized on the date shown above.

[redacted]

01/02/2017
Date

PROTOCOL ACCEPTANCE FORM

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**
PROTOCOL NUMBER:	ML29756 /ROC-BEV-2015-01
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MEDICINAL PRODUCT{S}:	Paclitaxel-Bevacizumab
SPONSOR:	Roche Farma S.A.

I agree to conduct the study in accordance with the current protocol.

Principal Investigator's Name (print)

Principal Investigator's Signature

Date

Please return the signed original of this form to the contact provided below. Please retain a copy for your study files.

[redacted]

Roche Farma. C/Eucalipto 33. 28035 Madrid.

PROTOCOL AMENDMENT, VERSION 2: JUSTIFICATION

The ML29756 study protocol has been modified in order to extend the recruitment period, until March 2017, as the inclusion period initially established in the protocol, and that finished one year after the inclusion of the first patient (9th December 2016), has not allowed us to reach the study's sample size.

At the same time, patients' follow up period has been reduced from 12 to 6 months after completing the treatment period in order not to delay the obtainment of clinical conclusions in the timelines initially set, due to the extension of the recruitment period, as the duration of the follow up period does not affect any of the study objectives.

To improve the quality and consistence, other minor changes have been performed to the protocol. Due to a mistake in the previous version, the definition of Clinical Benefit Rate has been modified. The significant information that has been added appears tracked in the version document in italics. This amendment shows the changes added to the original protocol.

PROTOCOL AMENDMENT, VERSION 2:
HISTORY OF CHANGES

PROTOCOL FINALIZATION SIGNATURE PAGE

[redacted], Roche Farma S.A

[redacted] ~~. Statistician~~
~~Date~~

SECTION 2: RESPONSIBLE PARTIES

[redacted], Roche Farma S.A.

SECTION 3: SYNOPSIS

Research Question and Objectives

Effectiveness Objectives

The primary effectiveness objective of this study is as follows:

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)

Study Design

Description of Study

…The development of the study involves the prospective collection of data for ~~24~~ *18* months after initiation of therapy.

... The expected bevacizumab dose will be 10 mg/~~M2~~ *kg* day 1 and 15 every four weeks.

… All patients will be followed for up to ~~24~~ *18* months from the start of treatment (including those who discontinue study treatment for any reason other than PD).

Variables

Primary Effectiveness Variable

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. …..

Statistical Considerations

Sample Size Justification

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)

Milestones

Study milestones are given in the following table.

Milestone	Planned Date
Start of data collection	Q42015
End of data collection	~~Q42018~~ *Q22018*
Final report of study results	~~Q42019~~ *Q22019*

Medicine

…The expected bevacizumab dose will be 10 mg/~~m~~^{2} *kg* day 1 and 15 every four weeks.

SECTION 5: MILESTONES

Milestone	Planned Date
Start of data collection	Q42015
End of data collection	~~Q42018~~ *Q22018*
Final report of study results	~~Q42019~~ *Q22019*

SECTION 7.2: 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)

SECTION 8.1.: STUDY DESIGN

…The development of the study involves the prospective collection of data for ~~24~~ *18* months after initiation of therapy.

SECTION 8.1.1: OVERVIEW OF STUDY DESIGN

…The development of the study involves the prospective collection of data for ~~24~~ *18* months after initiation of therapy. The total duration of the study comprise approximately ~~36~~ *33* months. Approximately, the inclusion will start in October 2015 and will end in ~~October 2016~~ *March 2017*. The last patient last visit is planned in ~~October 2018~~ *October 2019*. These dates may vary due to the approval process.

… The expected bevacizumab dose will be 10 mg/~~m~~^{2} *kg* day 1 and 15 every four weeks.

…All patients will be followed for up to ~~24~~ *18* months from the start of treatment (including those who discontinue study treatment for any reason other than PD).

SECTION 8.1.4.: Rationale for Study Design

… A prospective design is chosen where the CTC levels are obtained according the clinical practice before the initiation of first line treatment and patients are followed for ~~24~~ *18* months to evaluate the treatment response.

SECTION 8.2.: POPULATION

…Approximately, the inclusion will start in October 2015 and will end in ~~October 2016~~ *March 2017*.

SECTION 8.2.2.: Dosis, Administración, y Cumplimiento

…The expected bevacizumab dose will be 10 mg/~~m~~^{2} *kg* day 1 and 15 every four weeks.

SECTION 8.3.1.: Primary Effectiveness Variable

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….

SECTION 8.4.4: Data Collected During Follow-Up

The follow up period cover ~~one year~~ *approximately 6 months* after the last dose of study treatment.

SECTION 8.6.5.: Sample Size Justification

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)….

SECTION 8.8.3.: Administrative Structure

[redacted], Roche Farma S.A.

[redacted]~~. Statistician~~

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

1. LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event.
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBR	Clinical Benefit Rate
CI	Confidence Interval
CR	Complete Response
CRO	Contract Research Organization
CTC	Circulating Tumor Cells
CTCAE	Common Terminology Criteria for Adverse Events
EC	Ethics Committee
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
ENCePP	European Network of Centers for Pharmacoepidemiology and Pharmacovigilance
ePRO	Electronic Patient-Reported

Abbreviation	Definition
	Outcome
ER	Estrogen Receptor
FDA	Food and Drug Administration
FPFV	First Patient First Visit
GPP	Good Pharmacoepidemiological Practice
HER 2	Human Epidermal Growth Factor Receptor-Type2
IARC	International Agency for Research on Cancer
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
IRB	Institutional Review Board
ISPE	International Society of Pharmacoepidemiology
LDH	Lactate Dehydrogenase
LPLA	Last Patient Last Assessment
LPLV	Last Patient Last Visit
MBC	Metastatic Breast Cancer
NCI	National Cancer Institute
NPV	Negative Predictive Value
OR	Overall Response

Abbreviation	Definition
OS	Overall Survival
P	Progression
PDS	Pharma Development Safety
PFS	Progression-free Survival
PPV	Positive Predictive Value
PR	Partial Response
PR	Progesterone Receptor
QPPV	Qualified Person for Pharmacovigilance
RBC	Red blood cell
ROC	Receiver Operating Curve
SAE	Serious Adverse Event
SD	Stable Disease
SDV	Source Data Verification
SPC	Summary of Product Characteristics
SPSS	Statistical Package for the Social Sciences
STIAMP	Suspected Transmission of Infectious Agent by Medicinal Product
ULN	Upper Limit of Normal
WBC	White Blood Cell
WHO	World Health Organization

2. RESPONSIBLE PARTIES

[redacted]

[redacted], Roche Farma S.A.

Main Authors:

Dr. [redacted]

[redacted]. Tfno: [redacted]

Dr. [redacted]

[redacted]. Tfno: [redacted]

Dr. [redacted]

[redacted]. Tfno: [redacted]

Dr. [redacted]

[redacted]. Tfno: [redacted]

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3. SYNOPSIS

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**
PROTOCOL NUMBER:	ML29756 /ROC-BEV-2015-01
VERSION NUMBER:	2
DATE OF PROTOCOL:	16th December 2016
MEDICINAL PRODUCT:	Paclitaxel-Bevacizumab
INTERNATIONAL MEDICAL LEADER:	[redacted]
MAIN AUTHOR:	Dr. [redacted] Dr. [redacted] Dr. [redacted] Dr. [redacted]
PHASE:	IV, non-interventional study
INDICATION:	Metastasic breast cancer
SPONSOR:	Roche Farma S.A.

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.

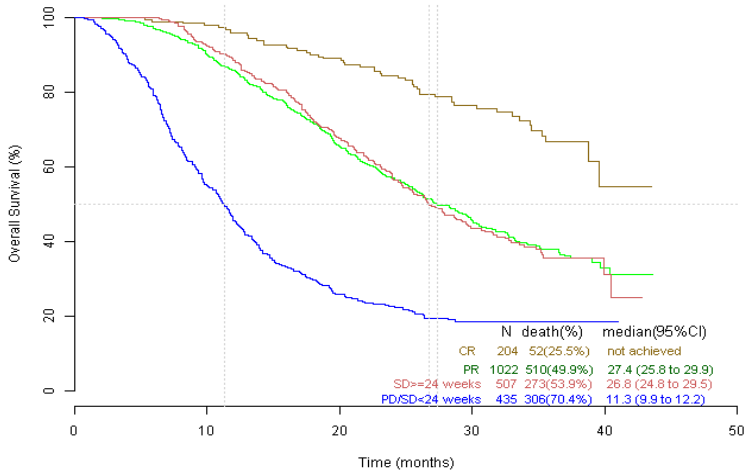
The main limitation of this study is that fails in making an early detection of the patients who do not benefit from the treatment with bevacizumab. Since the absence of efficacy was defined as progression within the first six cycles of

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 (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 $\geqslant$ 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.

Research Question and Objectives

Effectiveness Objectives

The primary effectiveness objective of this study is as follows:

- 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
- To evaluate the incidence of adverse events, serious adverse events and events of special interest in two prognostic groups and the total sample in routine clinical practice.

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)

Study Design

Description of Study

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).

Start Date of Study

The study start date will be the date of the first data collection: the date from which information on the first study subject is recorded in the study database or, in the case of secondary use of data, the date from which data extraction starts.

End of Study

The end of the study will be the date from which the last information of the last subject is recorded in the study database according to standard clinical practice.

Population

In this study, documentation of patients who are treated according to the local SmPC will be collected. Documentation of patients treated "off-label" is therefore excluded.

Patients must meet the following criteria for study entry:

- Patients aged ≥ 18 years
- Patients with HER2-negative MBC. Mandatory to have the HER2/estrogen receptor(ER)/progesterone receptor (PR) status
- Patient who met criteria for first-line treatment with chemotherapy plus bevacizumab (standard doses) by local, regional or national guidelines or authorities.
- Patients with measurable disease (RECIST criteria v1.1) or patients with no measurable but assessable disease.
- Molecular phenotype:
  - Triple negative metastatic breast cancer

- ER[+] tumors need to fulfill at least one of the two clinical criteria:
  - Endocrine resistance criteria: Metastatic relapse on adjuvant endocrine therapy or progression to at least one prior line of endocrine therapy for advanced disease or
  - Aggressive disease criteria (at least two criteria):
    - Taxane based regimen in the (neo)adjuvant setting.
    - Metastatic relapse within 2 years from the end of chemotherapy for early breast cancer.
    - Liver metastasis.
    - Three or more organs with metastatic involvement (skin - lymph - breast or soft tissue are all considered a single one).
    - Symptomatic visceral disease (patient not suitable for endocrine therapy).
- ECOG 0-2
- Signed informed consent form

Patients who meet any of the following criteria will be excluded from study entry:

- Patient has received prior chemotherapy for metastatic disease
- Patient requiring major/minor surgery within 3 weeks prior to administration of the first dose of study treatment
- Patient has received an investigational therapy within 4 weeks prior to study entry
- Patient has known symptomatic brain metastases.
- Patient with non measurable or assessable disease:
  - Exclusive blastic bone disease.
  - Pleural, pericardial or abdominal effusion as only evidence of disease.

- Patient in chronic daily treatment with corticosteroids (doses> 10 mg / day of methylprednisolone or equivalent), except inhaled steroids
- 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)
- Women of childbearing potential (defined as those who have had their last menstrual period <2 years and are not surgically sterilized) who are not using hormonal contraceptives or highly effective birth control (e.g. intrauterine device) during the study
- Patient has an active, uncontrolled bacterial, viral, or fungal infection(s) requiring systemic therapy
- Patient with significant renal, hematological or liver function alteration according to investigator's criteria
- Patient has serious medical risk factors involving any of the major organ systems

Variables

Only variables, obtained according to routine clinical pratice can and should be documented in this study.

Primary Effectiveness Variable

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 in one of the two determinations) and resistance group (≥ 5 CTCs in the two determinations)

Secondary Effectiveness Variable

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.

Safety Variables

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

Other Variables of Interest

Other variables of interest are depicted in the protocol.

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.

Study Size

The enrollment of 110 patients is planned for the study involving 30 sites throughout the country.

Statistical Considerations

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

Descriptive statistics and statistical plots will be used to describe all data. Categorical variables will be summarized with number of missing values, frequencies and percentages and exact binomial (Clopper-Pearson) confidence interval 95% (when necessary). Continuous variables will be summarized with the number of "non-missing" observations, the mean, standard deviation, median, interquartile range, minimum, maximum and 95% confidence interval (when necessary). Time to event variables will be summarized with rates and 95% confidence interval (whenever necessary).

Generally speaking, there are no plans to allocate unavailable data, which will simply be described as missing data. In addition, there are no plans to conduct a sensitivity analysis.

Analysis Methods

Primary Effectiveness Analysis

For the analysis of the primary objective we will compare CBR rates between CTC sensitive and resistance groups. The estimation will be performed with a Chi-square test for two independent proportions and presented together with 95% exact binomial (Clopper-Pearson) confidence intervals.

Secondary Effectiveness Analysis

We will estimate the positive predictive value (PPV), negative predictive value (NPV), sensibility and specificity of the prognostic value of CTC levels (two cut-offs points: <5 CTCs/7.5 ml and optimal cuts-off point) to detect patients who are either alive, without progression, with complete response or with objective response. The estimation will be performed with exact binomial (Clopper-Pearson) confidence interval 95% for proportions.

We will estimate the optimal cut off point for CTCs level with sample data for detecting patients who are either alive, without progression, with complete response or with objective response. Logistic regression models and receiver operating curve (ROC) procedures will be used.

We will estimate the differences between good and poor prognostic groups (defined by two cut-offs point: <5 CTC and optimal for sample) in overall survival, progression free survival and time to best response. Analysis will be adjusted by baseline prognostic factors. Cox regression models and time dependent ROC will be used.

Safety Analysis

Summary measures will be performed on the adverse events (adverse event, events of special interest, those which lead to a study or treatment discontinuation, SAEs and AEs grade ≥3)laboratory parameters and other security parameters. The incidence and the 95% CI of every event will be calculated and will be expressed with the primary system-organ class and the preferred term according to MedDRA version 17.1.

We will estimate the positive predictive value (PPV), negative predictive value (NPV), sensibility and specificity of the prognostic value of CTC levels (two cut-offs: <5 CTCs/7.5 ml and optimal cuts-off) to detect toxicities grade (3-4). The estimation will be performed with exact binomial (Clopper-Pearson) confidence interval 95% for proportions.

We will estimate the optimal cut off for CTCs level with sample data for detecting objective response and toxicities grade (3-4). Logistic regression models and receiver operating curve (ROC) procedures will be used.

Other Analyses

For the biomarkers correlation with the CTC levels, correlation coefficients (Pearson or Spearman depending on variable distribution) will be analyzed.

Interim Analyses

No interim analyses are planned.

Sample Size Justification

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 clinical benefit rate 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.

Milestones

Study milestones are given in the following table.

Milestone	Planned Date
Start of data collection	Q42015
End of data collection	Q22018
Final report of study results	Q22019

Medicine

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

4. PROTOCOL AMENDMENTS AND UPDATES

Any protocol amendments will be prepared by the Sponsor or designee.

Protocol amendments will be submitted to the Institutional Review Board (IRB)/Ethics Committee (EC) and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes that involve logistical or administrative aspects only (e.g., change in Study Monitor or contact information).

5. MILESTONES

Milestone	Planned Date
Start of data collection	Q42015
End of data collection	Q22018
Final report of study results	Q22019

6. RATIONALE AND BACKGROUND

6.1 BACKGROUND ON METASTATIC BREAST CANCER

Breast cancer is by far the most common female malignancy worldwide, with the highest rates reported in Europe and North America. (Parkin, D.M. et al. 2005) GLOBOCAN is a recent published report performed by the International Agency for Research on Cancer (IARC) the specialized bureau on cancer of World Health Organization. It states that breast cancer is the most frequent in women also in Spain (29% incidence and 40.8% prevalence), and the fourth most common type of cancer throughout the whole population (Fergay J, et al. 2012)

Breast cancer is increasingly being diagnosed at an early stage due to improved awareness and widespread screening programmes. In Spain, the 82.8% will survive after 5 years after diagnosis (Eurocare -4). The outlook for patients with metastatic breast cancer (MBC) is poor and the disease is generally not considered curable (National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology: Breast cancer v.2.2008). Median OS is around 24 months and the 5-year survival rate is low, at just 26 % (Jemal, A. et al, 2007*)*.

6.2 BACKGROUND ON PACLITAXEL-BEVACIZUMAB

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 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 use of bevacizumab in combination with paclitaxel was supported by the phase III clinical trial E2100 (Miller et al, 2007). This trial enrolled a total of 722 patients who were randomly assigned to receive 90 mg of paclitaxel per square meter of body-surface area on days 1, 8, and 15 every 4 weeks, either on its own or with 10 mg of bevacizumab per kilogram of body weight on days 1 and 15. The primary endpoint was PFS; OS was a secondary endpoint. Paclitaxel plus bevacizumab significantly prolonged PFS, compared with paclitaxel on its own (median 11.8 vs. 5.9 months; hazard ratio for progression 0.60; P<0.001) and increased the objective response rate (36.9% vs. 21.2%, p<0.001). The OS rate, however, was similar in the two groups (median 26.7 vs. 25.2 months; hazard ratio 0.88; p = 0.16).

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

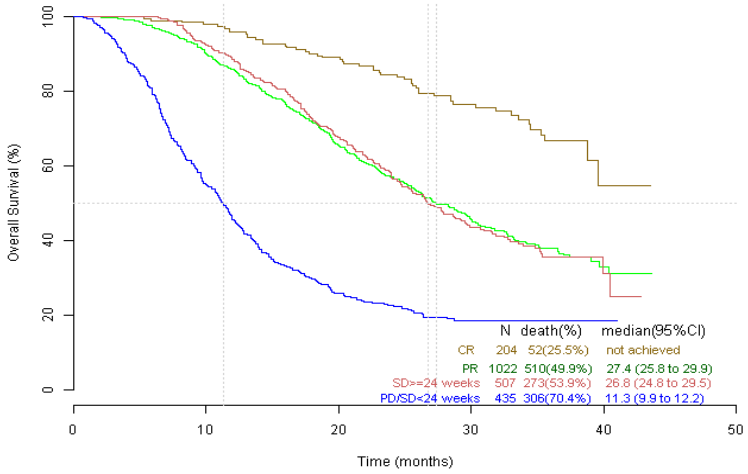
sixth cycle; and those that did not present progression criteria (stable disease or response) were randomized to maintenance with bevacizumab or to bevacizumab in combination with a second cytostatic (capecitabine), until disease progression or toxicity. The study shows a dramatic benefit in PFS (PFS: 4.3 vs. 11.9 months; Hazard ratio 0.38; p <.0001) and 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 (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.

6.3 STUDY RATIONALE

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 $\geqslant$ 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 (Smerage JB, et al. 2014) study has explored the ability of CTCs as prognostic and predictor markers leading to early treatment changes in patients with resistant criteria tumors. The study confirmed the ability of monitoring CTCs to predict early sensitivity to the first line treatment as well as prognosis. However, it failed in the primary objective of the study, as no difference in PFS or OS was observed when patients maintaining $\geqslant$ 5 CTCs after 3-5 weeks on therapy were randomized to maintain the first line therapy (n = 62; PFS/OS 3.5 and 10.7 months) or change to a non-cross-resistant regimen (n = 60; 4.6 and 12.5 months).

However, the SWOG S0500 study allows 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. The distribution was:

	% Patients	PFS	OS
Basall 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

The recently published work of Giuliano et al. (Giuliano et al. 2014) explores the path of the use of the CTCs value to predict the risk of tumor dissemination. This group has a dilated experience in the CTC levels investigation; in their retrospective study the data of 492 patients with advanced breast cancer who had the CTC count assessed levels prior to the new line treatment were analyzed. Their results suggest a new potential role of the CTC as an early predictor of the tumor's metastatic potential. Specifically, a pretreatment CTC level of ⩾5 CTCs/7.5 ml was associated with an increment in metastatic locations in comparison with <5 CTCs/7.5 ml levels (p=0.0077). Furthermore, at treatment failure, the patients with ≥5 CTCs/7.5 ml developed new metastatic lesions more frequently and in new locations in comparison with those with <5 CTCs/7.5 ml (development of new lesions, p=0.0002; lesions in new location, p=0.0031).

7. RESEARCH QUESTION AND OBJECTIVES

7.1 RESEARCH QUESTION

CTC monitoring could become a useful tool for early prediction of sensitivity/resistance to bevacizumab regimens. The SWOG 0500 trial has confirmed that there is no benefit from changing the cytotoxic agent or combination regimen 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. Furthermore, the recent works of Giuliano also state the predictive potential of the CTCs levels in tumor dissemination that has a high relation to the target of treatments such as bevacizumab.

Due to the aforementioned, our hypothesis is that early CTC monitoring, basal and after second cycle in patients initiating first line paclitaxel plus bevacizumab

chemotherapy, will identify two populations with very different progression free survival results; the population with maintained high CTC levels (≥ 5 CTCs in both determinations) will progress shortly, expecting a median PFS of 4 months, while the population with a low CTC determination (basal or after the second cycle) will achieve median PFS in the range of 13 months.

This study will identify a powerful and easy predictive/prognostic marker to use with patients under bevacizumab. The clinical implications of the IMELDA study will also help to increase the differences between both groups. For the study investigators it is important to state that this tool will optimize the treatment with the paclitaxel-bevacizumab combination, and this has important implications not only in patient management but in economic resources.

7.2 OBJECTIVES

Effectiveness Objectives

The primary effectiveness objective of this study is as follows:

- 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
- To evaluate the incidence of adverse events, serious adverse events and events of special interest in two prognostic groups and the total sample in routine clinical practice.

Safety Objectives

The safety objectives of 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)

8. RESEARCH METHODS

8.1 STUDY DESIGN

This is a multicenter, observational, prospective study in patients with MBC who initiate standard treatment with bevacizumab in combination with paclitaxel. The development of the study involves the prospective collection of data for18 months after initiation of therapy. The treatment decision is independent to study inclusion and in no case shall be subject to the patient's inclusion in the study. The visits will coincide patient's regular visits made to monitor his/her condition, without interfering in any way with the researcher's clinical practice.

To ensure the observational nature of the study, these data should always be collected, when available, from the medical record or they may be obtained during the interview with the subject in their regular visit, without any diagnostic or therapeutic interventions.

8.1.1 Overview of Study Design

The scope of the study covers the patients who are going to start a first line treatment with a combination of paclitaxel and bevacizumab. By this there are two medicines under observation, paclitaxel and bevacizumab. In both cases, the medicines will be used according to national labeling and local standard of care will be observed. The development of the study involves the prospective collection of data for 18 months after initiation of therapy. The total duration of the study comprise approximately 33 months. Approximately, the inclusion will start in October 2015 and will end in March 2017. The last patient last visit is planned in October 2019. These dates may vary due to the approval process.

It is planned to recruit a total of 110 patients from 30 Spanish sites.

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

1. 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).

A data collection overview is provided in Appendix 2.

8.1.2 Number of Patients Observed in the Study

It is planned to consecutively recruit a total of 110 patients.

8.1.3 Centers

This study will be conducted at approximately 30 centers in Spain.

Additional centers may be added or substituted if underperforming.

8.1.4 Rationale for Study Design

The present study assesses the predictive capacity of the CTC levels in the response to paclitaxel-bevacizumab treatment. A prospective design is chosen where the CTC levels are obtained according the clinical practice before the initiation of first line treatment and patients are followed for 18 months to evaluate the treatment response.

The disease is evaluated according to clinical practice and the response to treatment is assessed using the RECIST criteria v1.1, the gold standard of efficacy assessment in breast cancer. Clinical benefit, overall response, complete response, partial response, stable disease and progression will be calculated.

8.1.5 Rationale for Patient Population and Analysis Groups

Due to the primary objective of the study, it is necessary to include patients who have not yet received first line treatment to perform the CTC basal determination before any first line drug administration. The analysis groups will be created according to the CTC levels (5 CTCs/ 7.5 mL) and using a Receiver Operating Curve (ROC) curve for the secondary objective).

8.2 POPULATION

Patients with MBC receiving a combination treatment of paclitaxel-bevacizumab according to standard of care and in line with the current summary of product characteristics (SPC)/local labeling and who have no contraindication to paclitaxel-bevacizumab therapy as per the local label, are eligible for observation in this cohort if the following applies. The written informed consent form has to be signed before any study procedure. Approximately, the inclusion will start in October 2015 and will end in March 2017. Patients will be recruited from hospitals.

Patients must meet the following criteria for study entry:

- Patients aged ≥ 18 years
- Patients with HER2-negative MBC. Mandatory to have the HER2/estrogen receptor(ER)/progesterone receptor (PR) status

- Patient who met criteria for first-line treatment with chemotherapy plus bevacizumab (standard doses)by local, regional or national guidelines or authorities.

- Patients with measurable disease (RECIST criteria v1.1) or patients with no measurable but assessable disease.

- Molecular phenotype:

  - Triple negative metastatic breast cancer

  - ER[+] tumors need to fulfill at least one of the two clinical criteria:

    - ✓ Endocrine resistance criteria: Metastatic relapse on adjuvant endocrine therapy or progression to at least one prior line of endocrine therapy for advanced disease or

    - ✓ Aggressive disease criteria (at least two criteria):

      - Taxane based regimen in the (neo)adjuvant setting

      - Metastatic relapse within 2 years from the end of chemotherapy for early breast cancer

      - Liver metastasis

      - Three or more organs with metastatic involvement (skin - lymph - breast or soft tissue are all considered a single one)

      - Symptomatic visceral disease (patient not suitable for endocrine therapy)

  - ECOG 0-2.

  - Signed informed consent form

Patients who meet any of the following criteria will be excluded from study entry:

- Patient has received prior chemotherapy for metastatic disease

- Patient requiring major/minor surgery within 3 weeks prior to administration of the first dose of study treatment

- Patient has received an investigational therapy within 4 weeks prior to study entry

- Patient has known symptomatic brain metastases

- Patient with non measurable or assessable disease:
    - Exclusive blastic bone disease
    - Pleural, pericardial or abdominal effusion as only evidence of disease
- Patient in chronic daily treatment with corticosteroids (doses> 10 mg / day of methylprednisolone or equivalent), except inhaled steroids
- 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)
- Women of childbearing potential (defined as those who have had their last menstrual period <2 years and are not surgically sterilized) who are not using hormonal contraceptives or highly effective birth control (e.g. intrauterine device) during the study
- Patient has an active, uncontrolled bacterial, viral, or fungal infection(s) requiring systemic therapy
- Patient with significant renal, hematological or liver function alteration according to investigator's criteria
- Patient has serious medical risk factors involving any of the major organ systems

8.2.1 Concomitant Medication and Treatment

Concomitant medication prescribed for concomitant diseases of special interest and treatment for MBC at the beginning of the observation period or introduced during the observation period will be documented in the eCRF from the start of therapy with paclitaxel-bevacizumab until the end of the follow up if applicable.

8.2.2 Dosage, Administration, and Compliance

Dosing and treatment duration of the paclitaxel-bevacizumab are at the discretion of the investigator in accordance with local clinical practice and local labeling.

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.

8.3 VARIABLES

8.3.1 Primary Effectiveness Variable.

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 in one of the two determinations) and resistance group (≥ 5 CTCs in the two determinations)

8.3.2 Secondary 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.

8.3.3 Safety Variables

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

8.3.4 Other Variables of Interest

- 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)

8.4 TREATMENT EFFICACY EVALUATION DATA COLLECTION

8.4.1 Collection of Data on the CRF

Patients' medical records will be the source of all data that will be recorded in the CRFs. Therefore, only data available and already existing in patient's files will be recorded in CRFs. The degree of detail and completeness of data collected is dependent on what is recorded in the patient charts. Data from patient notes should be entered in the CRF as soon as they become available.

8.4.2 Data Collected during the Observation Period

During therapy with paclitaxel-bevacizumab, laboratory assessments routinely performed in accordance with current guidelines and local standard of care. When performed during the observational period, available results from the range of assessments described below will be documented on the CRF. Most data will be documented around the treatment period and some of them in the follow up period. The proposed assessments and suggested timings for assessments in the protocol/observational plan are not mandatory. It is up to the treating physician to perform and document the assessments as performed in the real clinical setting.

In the routine care setting, patients are seen regularly by their treating physicians either for treatment or for regular assessment after treatment. Thus, no study-specific visits or evaluations are required by this protocol.

Baseline visit

- Revision of the selection criteria
- Informed consent
- 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.

- CTC determination (baseline determination)
- 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).
- Treatment efficacy evaluation.

Treatment visits

These visits will coincide with a patient clinical appointment and are expected to be approximately each 9-12 weeks

- Clinical and pathological variables: concomitant medications, ECOG functional status, weight, first line therapy and toxicities that may occur during the study period
- CTC determination (a second determination of CTCs,in the 7 days prior to day 1/cycle 3 (before any treatment administration of cycle 3).)
- 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)
- 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)

Treatment efficacy evaluation. Please see Appendix 2 for the data collection overview (as per standard of care)

8.4.3 Data Collected at Study Completion/Early Termination Visit

For patients who complete the study, including early termination, the study completion visit should be documented.

Study completion visit

- Clinical and pathological variables: concomitant medications, ECOG functional status, weight, first line therapy and toxicities that may occur during the study period
- 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)
  - 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)

Treatment efficacy evaluation please see Appendix 2 for the data collection overview at the treatment completion visit if available.

8.4.4 Data Collected during Follow-Up

The follow up period cover approximately 6 months after the last dose of study treatment. The visits will coincide with a patient clinical appointment. The following information will be collected:

Follow up visit

Follow up visits will be performed after treatment discontinuation, due to progression or to other reason (patient/physician decision, toxicity, etc.). These visits will coincide with a patient clinical appointment and are expected to be approximately each 9-12 weeks

- Clinical and pathological variables: concomitant medications, ECOG functional status, weight, and toxicities that may occur during the study period
- 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)
    - 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).
- Treatment efficacy evaluation.

After the study completion/early termination visit, adverse events (AEs) should be followed as outlined in Section 10.5.

Please see Appendix 2 for the data collection overview during follow-up.

8.4.5 Safety Data Collection

Clinical AEs, serious and non-serious, will be recorded in the CRF throughout all the observation period, with physician's assessment of severity (mild, moderate, severe or in oncology studies using the National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE]) and relationship to therapy (i.e., related or unrelated) as described in Section 10.

8.4.6 Retrospective Data Collection

Not applicable

8.5 PATIENT, STUDY, AND SITE DISCONTINUATION

8.5.1 Patient Discontinuation

As the decision for treatment lies with the treating physician and is not bound to the participation of a patient in the study, the physician has the right to withdraw a patient from the study at any time. In addition, patients have the right to voluntarily withdraw from the study at any time for any reason. Reasons for discontinuation of treatment with the medicinal product or withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent at any time
- Physician or Sponsor judges discontinuation is in the patient's best interest
- Patient is lost to follow-up.
- Toxicity
- Progression.

8.5.2 Discontinuation from Treatment with Medicinal Product

The decision for discontinuation from treatment lies with the treating physician in accordance with the SmPC and in agreement with the patient's decision and is not regulated by this protocol.

The early termination visit should be completed by patients who discontinue treatment with paclitaxel or bevacizumab earlier than planned according to labeling. The primary reason for early treatment discontinuation should be documented on the appropriate CRF page. Every effort should be made to obtain information on patients who discontinue treatment.

8.5.3 Withdrawal from Study

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate CRF page. Patients will not be followed for any reason after consent has been withdrawn. Patients who withdraw from the study will not be replaced.

8.5.4 Study and Site Discontinuation

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

- Patient enrollment is unsatisfactory

The Sponsor will notify the physician if the study is placed on hold, or if the Sponsor decides to discontinue the study.

The Sponsor has the right to replace a site at any time. Reasons for replacing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the Guidelines for Good Pharmacoepidemiological Practices (GPP) or any other pertinent local law or guideline

8.6 STATISTICAL CONSIDERATIONS

8.6.1 General Considerations

Upon study completion, after the data for the final study patient have been recorded, the database will be closed and transferred to the Biometrics Department for statistical analysis.

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

Descriptive statistics and statistical plots will be used to describe all data. Categorical variables will be summarized with number of missing values, frequencies exact binomial (Clopper-Pearson) confidence interval 95% (when necessary). Continuous variables will be summarized with the number of "non-missing" observations, the mean, standard deviation, median, interquartile range, minimum, maximum and 95% confidence interval (when necessary). Time to event variables will be summarized with rates and 95% confidence interval (whenever necessary).

Generally speaking, there are no plans to allocate unavailable data, which will simply be described as missing data. In addition, there are no plans to conduct a sensitivity analysis.

8.6.2 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.

8.6.3 Analysis Methods

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

Descriptive statistics and statistical plots will be used to describe all data. Categorical variables will be summarized with number of missing values, frequencies and percentages and confidence interval 95% (when necessary). Continuous variables will be summarized with the number of "non-missing" observations, the mean, standard deviation, median, interquartile range, minimum, maximum and 95% confidence interval (when necessary). Time to event variables will be summarized with rates and 95% confidence interval (whenever necessary).

8.6.3.1 Primary Effectiveness Analysis

For the analysis of the primary objective an estimation using the Chi square for independent proportions and the 95% CI for each estimation of a group will be used and presented together with 95% exact binomial (Clopper-Pearson) confidence intervals. As a part of a secondary analysis the predictive values will be estimated to a new possible cutpoint.

8.6.3.2 Secondary Effectiveness Analysis

Secondary Effectiveness Analysis

We will estimate the positive predictive value (PPV), negative predictive value (NPV), sensibility and specificity of the prognostic value of CTC levels (two cut-offs points: <5 CTCs/7.5 ml and optimal cuts-off point) to detect patients who are either alive, without progression, with complete response or with objective response. The estimation will be performed with exact binomial (Clopper-Pearson) confidence interval 95% for proportions.

We will estimate the optimal cut off point for CTCs level with sample data for detecting patients who are either alive, without progression, with complete response or with objective response. Logistic regression models and ROC procedures will be used.

We will estimate the differences between good and poor prognostic groups (defined by two cut-offs point: <5 CTC and optimal for sample) in overall survival, progression free survival and time to best response. Analysis will be adjusted by baseline prognostic factors. Cox regression models and time dependent ROC will be used.

8.6.3.3 Safety Analysis

Summary measures will be performed on the adverse events (adverse event, events of special interest, those which lead to a study or treatment discontinuation, SAEs and AEs grade ≥3) laboratory parameters and other security parameters. The incidence and the 95% CI of every event will be calculated and will be expressed with the primary system-organ class and the preferred term according to MedDRA version 17.1.

We will estimate the positive predictive value (PPV), negative predictive value (NPV), sensibility and specificity of the prognostic value of CTC levels (two cut-offs: <5 CTCs/7.5 ml and optimal cuts-off) to detect toxicities grade (3-4). The estimation will be performed with exact binomial (Clopper-Pearson) confidence interval 95% for proportions.

We will estimate the optimal cut off for CTCs level with sample data for detecting objective response and toxicities grade (3-4). Logistic regression models and ROC procedures will be used.

8.6.3.4 Other Analyses

For the biomarkers correlation with the CTC levels, correlation coefficients (Pearson or Spearman depending on variable distribution) will be analyzed.

8.6.4 Interim Analyses

No interim analyses are planned.

8.6.5 Sample Size Justification

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.

8.7 DATA MANAGEMENT

8.7.1 Data Quality Assurance

The Sponsor will supply CRF specifications for this study. A contract research organization (CRO) will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected via EDC using CRFs. Sites will be responsible for data entry into the EDC system. In the event of

discrepant data, the CRO will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

The CRO will produce a Data Quality Plan that describes the quality checking to be performed on the data.

The Sponsor will perform oversight of the data management of this study, including approval of the CRO data management plans and specifications. Data will be periodically transferred electronically from the CRO to the Sponsor, and the Sponsor standard procedures will be used to handle and process the electronic transfer of these data.

CRFs and correction documentation will be maintained in the EDC system audit trail. System backups for data stored at the CRO and records retention for the study data will be consistent with the CRO standard procedures.

8.7.2 Electronic Case Report Forms

CRFs are to be completed using a Sponsor-designated electronic data capture (EDC) system. Sites will receive training and have access to a manual for appropriate CRF completion. CRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

All CRFs should be completed by designated trained site staff. CRFs should be reviewed and electronically signed and dated by the physician or a designee.

At the end of the study, the physician will receive the data related to patients from his or her site in an electronically readable format (e.g., on a compact disc [CD]). Data must be kept with the study records. Acknowledgement of receipt of the data is required.

8.7.3 Source Data Documentation

Study monitors will perform ongoing SDV as defined in the Trial Monitoring Plan to confirm that critical protocol data (i.e., source data) entered in the CRF by authorized site personnel are accurate, complete, and verifiable from source documents.

Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, PROs, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that contain study-relevant information will be clearly defined in the Trial Monitoring Plan. This includes any protocol data to be entered directly into the CRF (i.e., no prior written or electronic record of the data) and considered source data.

Source documents that are required to verify the validity and completeness of data entered in the CRF must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 8.7.5.

To facilitate SDV, the physicians and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The participating sites must also allow inspection by applicable health authorities.

8.7.4 Use of Computerized Systems

When clinical observations are entered directly into a participating site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with health authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

8.7.5 Retention of Records

Records and documents pertaining to the conduct of this study, including CRFs and Informed Consent Forms, must be retained by the Physician for at least 15 years after completion or discontinuation of the study, or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8.8 STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

8.8.1 Study Documentation

The physician must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval. In addition, at the end of the study, the physician will receive

the patient data, which include an audit trail containing a complete record of all changes to data.

8.8.2 Site Audits and Inspections

Site visits will be conducted by the Sponsor or an authorized representative for audit of study data, patients' medical records, and CRFs.

The physician will also permit national and local health authorities to inspect facilities and records relevant to this study.

8.8.3 Administrative Structure

Main Authors:

Dr. [redacted], MD. [redacted].

Dr. [redacted], MD. [redacted].

Dr. [redacted], MD. [redacted].

Dr. [redacted], MD. [redacted].

[redacted]. [redacted], Roche Farma S.A.

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8.9 LIMITATIONS OF THE RESEARCH METHOD

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

8.10 OTHER ASPECTS

No other aspects are worth to mention.

9. PROTECTION OF HUMAN SUBJECTS/ETHICAL CONSIDERATIONS

9.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the Guidelines for GPP published by the International Society of Pharmacoepidemiology (ISPE) and the laws and regulations of the country in which the research is conducted.

9.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's Assent or Caregiver's Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the

local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate Consent Forms proposed by the site (collectively, the “Consent Forms”) before IRB/EC submission. The final Consent Forms approved by the IRB/EC must be provided to the Sponsor for health authority submission purposes according to local requirements.

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

By signing the form, the patient confirms that he/she has been informed about the study and agrees to pseudonymous data collection, pooling of data with similar scientific data (if applicable), and the possibility of monitoring activities. It is the responsibility of the physician to obtain written informed consent from each patient participating in the study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. The physician must also explain that the patient is completely free to refuse to enter the study or to withdraw from it at any time, for any reason and without losing the benefit of any medical care to which the patient is entitled or is presently receiving.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient’s legally authorized representative. All signed and dated Consent Forms must remain in each patient’s study file or in the site file and must be available for verification by study monitors at any time.

9.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC by the Lead Scientific Responsible and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC.

The Lead Scientific Responsible is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Physicians are also responsible for promptly informing the IRB/EC of any protocol amendments.

In addition to the requirements for collecting all AEs in the CRF and reporting all suspected ADRs (including causality unknown or not provided), adverse events of special interest, and SAEs to the Sponsor, physicians must comply with requirements for AE reporting to the local health authority and IRB/EC.

9.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in datasets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

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

10. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ ADVERSE REACTIONS

10.1 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording SAEs and non-serious AEs (including AEs of special interest), performing safety laboratory assessments, measuring vital signs, and conducting other tests that are deemed critical to the safety evaluation of the study as per standard medical practice. For details of the monitoring and recording of AEs, please see Sections 10.2 and 10.3. Situations requiring safety reporting possibly without an associated AE are outlined in Section 10.3.4.

Certain types of events require immediate reporting to the Sponsor, as outlined in Section 10.3.

10.1.1 Adverse Events

According to the International Conference of Harmonisation (ICH), an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition), except as described in Section 10.2.4.10.
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram [ECG], X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study medicine.

10.1.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

An SAE is any AE that meets any of the following criteria:

- Is fatal (i.e., the AE actually causes or leads to death)
- Is life-threatening (NOTE: The term "life-threatening" refers to an event in which the patient was at immediate 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 or prolongs inpatient hospitalization (see Section 10.2.4.11)
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study medicine
- Is a significant medical event in the physician's judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are <u>not</u> synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to NCI CTCAE criteria; see Section 10.2.2); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each AE recorded on the CRF.

SAEs are required to be reported by the physician to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 10.3.1 and Section 10.3.2 for reporting instructions).

10.1.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Non-serious AEs of special interest are required to be reported by the physician to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 10.3.1 and Section 10.3.2 for reporting instructions). AEs of special interest for this study include the following:

- Cases of potential medicine-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law (see Section 10.2.4.7).
- Suspected transmission of an infectious agent by the study medicine, as defined below:

  Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term only applies when a contamination of the study medicine is suspected.
- Hypertension ≥ grade 3
- Proteinuria ≥ grade 3
- GI perforation, abscesses and fistulae (any grade)
- Wound healing complications ≥ grade 3
- Haemorrhage ≥ grade 3 (any grade CNS bleeding; ≥ grade 2 haemoptysis)
- Arterial thromboembolic events (any grade)
- Venous thromboembolic events ≥ grade 3
- PRES (or RPLS; any grade)
- Congestive heart failure ≥ grade 3
- Non-GI fistula or abscess ≥ grade 2Arterial thromboembolic events, congestive heart failure, interstitial lung disease, osteonecrosis of jaw

10.1.4 Non-Serious Adverse Events other than Adverse Events of Special Interest

All non-serious AEs (in addition to AEs of special interest) will be collected for this study.

10.2 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The physician is responsible for ensuring that all AEs (see Section 10.1.1 for definition) are recorded on the AE CRF and reported to the Sponsor in accordance with instructions provided in this section and in Section 10.3• Section 10.5.

For each AE recorded on the AE CRF, the physician will make an assessment of seriousness (see Section 10.1.2 for seriousness criteria), severity (see Section 10.2.2), and causality (see Section 10.2.3).

10.2.1 Adverse Event Reporting Period

Physicians will seek information on AEs at each patient contact. All AEs subject to the collecting and reporting requirements outlined in this protocol, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record and on the AE CRF.

After initiation of the study, all protocol-defined AEs will be reported from the time a patient enters the study until the end of his or her observation period. After this period, the physician is not required to actively monitor patients for AEs; however, the Sponsor should be notified if the physician becomes aware of any AEs, SAEs, AESIs or non-serious AEs of special interest related to the study medicine (see Section 10.5).

10.2.2 Assessment of Severity of Adverse Events

The AE severity grading scale for the NCI CTCAE (v 4.0) will be used for assessing AE severity. The table below will be used for assessing severity for AEs that are not specifically listed in the NCI CTCAE.

Table 1 Adverse Event Severity Grading Scale

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living [a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living [b, c]
4	Life-threatening consequences or urgent intervention indicated [d]
5	Death related to AE [d]

Note: Based on the NCI CTCAE (v 4.0), which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

[a] Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[b] Examples of self-care activities of daily living include bathing, dressing and undressing, feeding oneself, using the toilet, and taking medications, as performed by patients who are not bedridden.

[c] If an event is assessed as a "significant medical event," it must be reported as an SAE (see Section 10.3.2 for reporting instructions), per the definition of SAE in Section 10.1.2.

[d] Grade 4 and 5 events must be reported as SAEs (see Section 10.3.2 for reporting instructions), per the definition of SAE in Section 10.1.2.

10.2.3 Assessment of Causality of Adverse Events

Physicians should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an AE is considered to be related to the study medicine, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration:

- Temporal relationship of event onset to the initiation of study medicine
- Course of the event, considering especially the effects of dose reduction, discontinuation of study medicine, or reintroduction of study medicine (when applicable)
- Known association of the event with the study medicine or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event

For patients receiving combination therapy, causality will be assessed individually for each of the medicinal product.

10.2.4 Procedures for Recording Adverse Events

Physicians should use correct medical terminology/concepts when recording AEs in the AE section of the CRF. Colloquialisms and abbreviations should be avoided.

Only one AE term should be recorded in the event field of the CRF.

10.2.4.1 Infusion-Related or Injection Reactions

AEs that occur during or within 24 hours after study medicine administration and are judged to be related to study medicine infusion ***or*** injection should be captured as a diagnosis (e.g., infusion-related reaction ***or*** injection-site reaction ***or*** anaphylactic reaction) in the AE section of the CRF. If possible, avoid ambiguous terms such as "systemic reaction." Associated signs and symptoms should be recorded on the dedicated infusion-related reaction ***or*** injection reaction CRF. If a patient experiences both a local and systemic reaction to the same dose of study medicine, each reaction should be recorded separately in the AE section of the CRF, with signs and symptoms also recorded separately on the dedicated infusion-related reaction *or* injection reaction CRF.

10.2.4.2 Diagnosis versus Signs and Symptoms

For AEs other than infusion-related or injection reactions (see Section 10.2.4.1), a diagnosis (if known) should be recorded in the AE section of the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded in the AE section of the CRF. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by one AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

10.2.4.3 Adverse Events Occurring Secondary to Other Events

In general, AEs that are secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. A medically significant secondary AE that is separated in time from the initiating event should be recorded as an independent event in the AE section of the CRF. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the CRF.
- If vomiting results in severe dehydration, both events should be reported separately on the CRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the CRF.

- If dizziness leads to a fall and subsequent fracture, all three events should be reported separately on the CRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the CRF.

All AEs should be recorded separately in the AE section of the CRF if it is unclear as to whether the events are associated.

10.2.4.4 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation timepoints. Such events should only be recorded once in the AE section of the CRF. The initial severity (intensity or grade) of the event will be recorded at the time the event is first reported. If a persistent AE becomes more severe, the most extreme severity should also be recorded in the AE section of the CRF. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 10.3.1 and Section 10.3.2 for reporting instructions). The AE section of the CRF should be updated by changing the event from "non-serious" to "serious," providing the date that the event became serious, and completing all data fields related to SAEs.

A recurrent AE is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an AE should be recorded separately in the AE section of the CRF.

10.2.4.5 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an AE. A laboratory test result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Is clinically significant in the physician's judgment

It is the physician's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 • the upper limit of normal [ULN] associated with cholestasis), only the diagnosis (i.e., cholestasis) should be recorded in the AE section of the CRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded in the AE section of the CRF, along with a descriptor indicating if the test result is above or below the normal range (e.g., "elevated potassium," as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the AE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should only be recorded once in the AE section of the CRF (see Section 10.2.4.4 for details on recording persistent AEs).

10.2.4.6 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an AE. A vital sign result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Is clinically significant in the physician's judgment

It is the physician's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an AE.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (i.e., hypertension) should be recorded in the AE section of the CRF.

Observations of the same clinically significant vital sign abnormality from visit to visit should only be recorded once in the AE section of the CRF (see Section 10.2.4.4 for details on recording persistent AEs).

10.2.4.7 Abnormal Liver Function Tests

The finding of an elevated ALT or AST (> 3 • the ULN) in combination with either an elevated total bilirubin (> 2 • the ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of severe liver injury. Therefore, physicians must report as an AE the occurrence of either of the following:

- Treatment-emergent ALT or AST > 3 • ULN in combination with total bilirubin > 2 • the ULN
- Treatment-emergent ALT or AST > 3 • ULN in combination with clinical jaundice

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded in the AE section of the CRF (see Section 10.2.4.2) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event) either as an SAE or a non-serious AE of special interest (see Section 10.3.1 and Section 10.3.2).

10.2.4.8 Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 10.2.1), regardless of relationship to study medicine, must be recorded in the AE section of the CRF and immediately reported to the Sponsor (see Section 10.3.1 and Section 10.3.2).

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the AE section of the CRF. Generally, only one such event should be reported. The term **"sudden death"** should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without preexisting heart disease, within 1 hour after the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable. If the cause of death is unknown and cannot be ascertained at the time of reporting, **"unexplained death"** should be recorded on the AE section of the CRF. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death.

10.2.4.9 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions section of the CRF.

A preexisting medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events in the AE section of the CRF, it is important to convey the concept that the

preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

10.2.4.10 Lack of Therapeutic Efficacy or Worsening of Metastatic Breast Cancer

Events that are clearly consistent with the expected pattern of progression of the underlying disease should not be recorded as AEs. These data will be captured as effectiveness assessment data only. In most cases, the expected pattern of progression will be based on RECIST criteria. In rare cases, the determination of clinical progression will be based on symptomatic deterioration. However, every effort should be made to document progression through use of objective criteria. If there is any uncertainty as to whether an event is due to disease progression, it should be reported as an AE. This exception from reporting includes events of disease progression with a fatal outcome which are clearly attributable to disease progression.

10.2.4.11 Hospitalization or Prolonged Hospitalization

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE (per the definition of SAE in Section 10.1.2), except as outlined below.

The following hospitalization scenarios are not considered to be SAEs:

- Hospitalization for respite care
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
  - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
  - The patient has not suffered an AE.
- Hospitalization due solely to progression of the underlying cancer

The following hospitalization scenarios are not considered to be SAEs but should be reported as AEs instead:

- Hospitalization that was necessary because of patient requirement for outpatient care outside of normal outpatient clinic operating hours

10.2.4.12 Overdoses, Misuses, Abuses, Off-Label Use, Occupational Exposure, or Medication Error

Any overdose, misuse, abuse, off-label use, occupational exposure, medication error, or any other incorrect administration of medicine under observation should be noted on the Drug Administration section of the CRF. Any overdose, abuse, misuse, inadvertent/erroneous administration, medication error, or occupational exposure reports must be forwarded to the sponsor with or without an AE.

Reports with or without an AE should be forwarded to the Sponsor as per non-serious timelines. If the associated AE fulfills the seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event, see Section 10.3.1 and Section 10.3.2).

For the purpose of reporting cases of suspected adverse reactions, an occupational exposure to a medicine means an exposure to a medicine as a result of one's professional or non-professional occupation.

10.3 REPORTING REQUIREMENTS FROM PHYSICIAN TO SPONSOR

10.3.1 Immediate Reporting Requirements from Physician to Sponsor

Certain events require immediate reporting to allow the Sponsor/Marketing Authorization Holder and the regulatory authorities to take appropriate measures to address potential new risks associated with the use of the medicine. The physician must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the physician learns of the event. The following is a list of events that the physician must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study medicine:

- SAEs
- Non-serious AEs of special interest
- Pregnancies

The physician must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Physicians must also comply with local requirements for reporting SAEs to the local health authority and IRB/EC.

10.3.2 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

For reports of SAEs and non-serious AEs of special interest, physicians should record all case details that can be gathered immediately (i.e., within 24 hours) in the AE page of the CRF and submit the report via the EDC system. A report will be generated and sent to Roche Safety Risk Management by the EDC system.

In the event that the EDC system is temporarily unavailable, please refer to Section 10.7.

10.3.3 Reporting Requirements for Non-Serious Adverse Drug Reactions

For non-serious AEs considered related to any study medicine (including AEs for which causality is unknown or not assessed), physicians must record all case details that can be gathered within 30 calendar days of learning of the event on the AE section of the CRF and submit the report via the EDC system. A report will be generated and sent to Roche Safety Risk Management to allow appropriate reporting to relevant competent authorities.

In the event that the EDC system is temporarily unavailable, please refer to Section 10.7.

Non-serious AEs that are suspected to be related only to medicinal products other than the studied medicine should be reported by the physician/consumer to the marketing authorization holder of the suspected medicinal product, or to the concerned competent authorities via the national spontaneous reporting system.

10.3.4 Reporting Requirements for Pregnancies/Breastfeeding

10.3.4.1 Pregnancies/Breastfeeding in Female Patients

Female patients of childbearing potential will be instructed to immediately inform the physician if they become pregnant during the study or within 90 days after the end of the treatment after the last dose of medicine. A Pregnancy Report CRF (if available) should be completed by the physician immediately (i.e., no more than 24 hours after learning of the pregnancy) and submitted via the EDC system. A pregnancy report will automatically be generated and sent to Roche Safety Risk Management. Pregnancy should not be recorded on the AE CRF. The physician should discontinue the medicine and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any SAEs associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the AE section of the CRF.

In the event that the EDC system is temporarily unavailable please refer to Section 10.7.

Suspected adverse reactions that occur in infants following exposure to a medicinal product from breast milk should be reported to Roche Safety Risk Management.

10.3.4.2 Pregnancies in Female Partners of Male Patients

Male patients will be instructed through the Informed Consent Form to immediately inform the physician if their partner becomes pregnant during the study or within within 174 days after the end of the treatment after the last dose of study medicine. A Pregnancy Report CRF should be completed by the physician immediately (i.e., no more than 24 hours after learning of the pregnancy) and submitted via the EDC system. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male patient exposed to study medicine. The pregnant partner will need to sign an Authorization for Use and Disclosure of Pregnancy Health

Information to allow for follow-up on her pregnancy. Once the authorization has been signed, the physician will update the Pregnancy Report CRF with additional information on the course and outcome of the pregnancy. An physician who is contacted by the male patient or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

In the event that the EDC system is temporarily unavailable, please refer to Section 10.7.

10.3.4.3 Abortions

Any abortion should be classified as an SAE (as the Sponsor considers abortions to be medically significant), recorded in the AE section of the CRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 10.3.1 and Section 10.3.2).

10.3.4.4 Congenital Anomalies/Birth Defects

Any congenital anomaly/birth defect in a child born to a female patient exposed to the medicine or the female partner of a male patient exposed to the medicine should be classified as an SAE, recorded in the AE section of the CRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 10.3.1 and Section 10.3.2).

10.4 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

10.4.1 Physician Follow-Up

The physician should follow each AE until the event has resolved to baseline grade or better, the event is assessed as stable by the physician, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to study medicine or trial-related procedures until a final outcome can be reported.

During the study period, resolution of AEs (with dates) should be documented in the AE section of the CRF and in the patient's medical record to facilitate SDV.

All pregnancies reported during the study should be followed until pregnancy outcome. If the EDC system is not available at the time of pregnancy outcome, follow reporting instructions provided in Section 10.7.

10.4.2 Sponsor Follow-Up

For SAEs, non-serious AEs of special interest , and pregnancies , the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

10.5 POST-STUDY ADVERSE EVENTS

At the study completion/early termination visit, the physician should instruct each patient to report to the physician any subsequent AEs that could be related or not to the medicine. The physician should notify the Sponsor of any death, SAE, AEs or non-serious AE of special interest related to the medicinal product occurring at any time after a patient has discontinued study participation. The Sponsor should also be notified if the physician becomes aware of the development of cancer or a congenital anomaly/birth defect in a subsequently conceived offspring of a patient that participated in this study. The physician does not need to actively monitor patients for AEs once the trial has ended.

The physician should report these events directly to Roche or its designee, either by faxing or by scanning and emailing the Serious Adverse Event / Adverse Event of Special Interest Reporting Form using the fax number or email address provided to physicians.

10.6 EXPEDITED REPORTING TO HEALTH AUTHORITIES, PHYSICIANS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all SAEs and non-serious AEs of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to physicians, IRBs, ECs, and applicable health authorities based on applicable legislation.

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

- Local prescribing information for paclitaxel and bevacizumab
- Paclitaxel and bevacizumab Core Data Sheet

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

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

Certain AEs are anticipated to occur in the study population at some frequency independent of exposure to the medicine and will be excluded from expedited reporting. These anticipated events include disease progression as is described in 10.2.4.10.

10.7 IF EDC SYSTEM IS TEMPORARILY UNAVAILABLE OR NOT USED

In the event that the EDC system is temporarily unavailable, a completed paper reporting form (gcp_for003008) and fax coversheet should be faxed/scanned to Roche Safety Risk Management or its designee immediately (i.e., no more than 24 hours after

learning of the event) or within 30 days for non-serious ADRs if not AEs of special interest, using the fax number or email address provided to physicians.

In the event that the EDC system is temporarily unavailable, the Clinical Trial Pregnancy Reporting Form (gcp_for000023) provided to physicians should be completed and submitted to Roche or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy).

Once the system is available again, all information should additionally be entered and submitted via the EDC system.

11. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, both at scientific congresses and in peer-reviewed journals. The Sponsor will comply with all requirements for publication of study results. For more information, refer to the Roche Global Policy on Sharing of Clinical Trials Data at the following Web site:

http://www.rochetrials.com/pdf/RocheGlobalDataSharingPolicy.pdf

The results of this study may be published or presented at scientific meetings. If this is foreseen, the physician must agree to submit all manuscripts or abstracts to the Sponsor prior to submission for publication or presentation. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the physician.

In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating physician will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors' authorship requirements. Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the physician and the appropriate Sponsor personnel.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

To ensure the equitable site representativeness, the authors order will be according the number patients recruited in each centre.

The manuscript will included the maximum number of authors permitted by the Journal.

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Appendix 1
List of Stand-Alone Documents Not Included in the Protocol

- List of contact details of responsible parties and all physicians

Appendix 2
Data Collection Overview (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.

Appendix 3 Procedures of extraction, processing and shipment of samples of Circulating Tumor Cells (CTCs)

The CTCs determination will be performed by Cell Search system. During the start up visit and along the study, it will be given specific tubes to the sites for the samples for CTCs determination. The blood drawing have to be EXCLUSIVELY made in the Cell Save tubes given. Never have to be used other types of tubes.

The tube for CTCs is of 10 ml. To perform the draw is must be a minimum blood volume of 7.5 ml. Usually, if there is not any problem, a volume of approximately 8.5 ml is taken. If the blood drawing is complicated, it can blend the volume from some tubes, up to 7.5 ml.

If blood sample is taken in a multi-sample tube holder (Vacutainer, etc.) the Cell Save tube NEVER HAVE TO BE THE FIRST IN BE REFILLED. Refill others before the Cell Save tube. If only is going to be taken the sample for the CTCs, rule out, in other tube, the first 3-4 ml of the sample.

Once the sample is taken, is must be sent (without be centrifuged or be cooled) to the reference laboratory or local (only if the site has the CellSearch system)

The samples must be sent by courier service, as soon as possible, because have to be processed in a maximum of 96 hours after the draw (extractions on Friday must be avoid)

The samples will be sent to the reference laboratories, by no urgent courier service. Contact data will be provided at start up visit.

Important information:

- The eCRF must registry if the sample have been draw using a central or peripheral via.
- The Cell Search tubes given to the sites will have a referral note with a phone number for consultancy, if the person who makes the extraction has any doubt.
- In the tubes must be write down the patient code gives by the eCRF.

The determination will be carried out in two reference laboratories ([redacted]) to manage the number of received samples and have the most homogenous results.

All costs derived by study determinations are defrayed by the study sponsor.