PROTOCOL TITLE

“Neoadjuvant chemotherapy with nab-paclitaxel in women with HER2-negative high-risk breast cancer“

ETNA (Evaluating Treatment with Neoadjuvant Abraxane)

EudraCT number 2012-003481-41
Protocol number FM-12-B01
Amendment No. 1
Type: Substantial

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Acronym (if available) : ETNA (Evaluating Treatment with Neoadjuvant Abraxane)
Substantial Amendment No.1
Protocol Version 2.0 of 10th March 2014

"Neoadjuvant chemotherapy with nab-paclitaxel in women with HER2-negative high-risk breast cancer"

PROTOCOL APPROVAL

<table>
<thead>
<tr>
<th>Sponsor Study Chairman</th>
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</thead>
<tbody>
<tr>
<td>Dr. LUCA GIANNI</td>
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<td>Signature:</td>
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<table>
<thead>
<tr>
<th>Signature Date:</th>
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<td>10/03/2014</td>
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## Protocol Amendment History

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Study synopsis</td>
<td>Stratification variables will include Cooperative Research Group Disease stage [operable (tumor stage: T2N0-1; T3N0) and locally advanced (T3N1; T4, any N2-3)] Tumor subtype [luminal B intermediate (HER2 negative, ER or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER or PGR positive, Ki67 &gt;20%) vs triple negative tumors (HER2 negative, ER negative and PGR negative, Ki67 any value)]</td>
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<td>To better describe locally advanced disease and tumor subtype</td>
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<tr>
<td>Study design</td>
<td></td>
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<td></td>
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<tr>
<td>Eligibility criteria</td>
<td>6. One of the following clinical stages: a.T2, T3, T4 disease, triple negative (HER2, ER, PGR) b.T2, T3, T4 disease, ER or PGR positive and moderately differentiated or poorly differentiated tumor grade (G II-III)</td>
<td>6. One of the following breast cancer stages: a.T2, T3, T4-a-d disease, triple negative (HER2, ER, PGR) regardless of Ki67 value b.T2, T3, T4-a-d disease, ER and/or PGR positive. If Ki67 can be performed at the site, local Ki67 value must be ≥ 14%. If Ki67 is not available at the site, the tumor grade must be assessed as grade 2 or 3</td>
<td>To clarify that all T4 lesions, including inflammatory cancer, are eligible and that patients with ER and/or PGR positive disease are eligible only if local Ki67 value is ≥ 14% or, if Ki67 is not assessed locally, tumor grade must be assessed as grade 2 or 3</td>
</tr>
<tr>
<td>Main Protocol</td>
<td>The stratification variables will be: Cooperative Research Group Disease stage [operable (tumor stage: T2N0-1; T3N0) and locally advanced (T3N1; T4, any N2-3)] Tumor subtype [luminal B intermediate (HER2 negative, ER or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER or PGR positive, Ki67 &gt;20%) vs triple negative tumors (HER2 negative, ER negative and PGR negative, Ki67 any value)]</td>
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<tr>
<td>Section 3</td>
<td>Study design, Figure 2, right</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Core Biopsy after 1st cycle of Abraxane/ Paclitaxel (Recommended)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Core Biopsy before the start of the second cycle of ABX/PTX between day 21 and day 24 (Recommended)</td>
</tr>
<tr>
<td>Section 3</td>
<td>Study design, Figure 2, bottom</td>
<td>Not present</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Section 4</td>
<td>Paragraph 4.3</td>
<td>The treatment assigned will be based on a stratified randomization procedure using Cooperative Research Group; Disease stage [operable (tumor stage: T2N0-1; T3N0) and locally advanced (T3N1; T4, any N2-3)]; Tumor subtype [luminal B intermediate (HER2 negative, ER or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER or PGR positive, Ki67 &gt;20%) vs triple negative tumors (HER2 negative, ER negative and PGR negative, Ki67 any value)].</td>
<td>The treatment assigned will be based on a stratified randomization procedure using Cooperative Research Group; Disease stage [operable (tumor stage: T2N0-1; T3N0) and locally advanced (T3N1; T4 any N; any T N2-3)]; Tumor subtype [luminal B intermediate (HER2 negative, ER and/or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER and/or PGR positive, Ki67 &gt;20%) vs triple negative tumors (HER2 negative, ER negative and PGR negative, Ki67 any value)].</td>
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<tr>
<td>Section 5</td>
<td>Paragraph 5.2</td>
<td>6. One of the following clinical stages: a. T2, T3, T4 disease, triple negative (HER2, ER, PGR) b. T2, T3, T4 disease, ER or PGR positive and moderately differentiated or poorly differentiated tumor grade (G II-III)</td>
<td>6. One of the following breast cancer stages: a. T2, T3, T4a-d disease, triple negative (HER2, ER, PGR) regardless Ki67 value b. T2, T3, T4a-d disease, ER and/or PGR positive. If Ki67 can be performed at the site, local Ki67 value must be ≥ 14%. If Ki67 is not available at the site, the tumor grade must be assessed as grade 2 or 3</td>
</tr>
<tr>
<td>Section 6</td>
<td>Paragraph 6.1</td>
<td>The result of the referral laboratory will be made available on e-CRF and a hard copy of the appropriate page, which also includes UPN, stratification variable and patient eligibility, will be faxed to the study site for filing in the patient’s medical record as source document, and to the Central Office of the Cooperative Research Group.</td>
<td>The result of the referral laboratory will be recorded on the appropriate section of the e-CRF. A hard copy of the appropriate page, which also includes UPN, will be sent periodically to the study site for filing in the patient’s medical record as source document, and to the Central Office of the Cooperative Research Group.</td>
</tr>
<tr>
<td>Section 7</td>
<td>Paragraph 7.1</td>
<td>All patients must provide written informed consent before any study specific assessments or procedures are performed. However, investigations performed as part of the patient’s routine care before she has</td>
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</tr>
<tr>
<td>Section 7</td>
<td>Primary tumor core biopsy (see instructions for taking core biopsies in the Michelangelo ETNA Guidelines)</td>
<td>Not present</td>
<td>Already mentioned in previous paragraph</td>
</tr>
</tbody>
</table>

Consented to the study, may be used as study assessments and do not need to be repeated as long as they fall within the required time window of 3 weeks before registration.

(including HER2 status, ER and PgR status, and Ki67 value and/or tumor grade) is available to the site. All screening procedures must be performed within a maximum of 4 weeks from the date of signature of Informed Consent and all eligibility criteria must be entered by the site into the e-CRF for evaluation of patient eligibility by Michelangelo.

Once all the screening data and the local pathology report data had been entered in the e-CRF, the tumor block required for central laboratory review, must be shipped to the referral laboratory. If the sample shipped to the referral laboratory is insufficient and not representative of the invasive tumor component, the patient will be informed that unfortunately the tumor sample was inadequate for assessment and the patient can either (1) Undergo a further breast biopsy, which if sufficient and meets inclusion criteria will enable the patient to be randomized onto trial; or (2) Not proceed with a further breast biopsy and be regarded as ineligible for the clinical trial (i.e. screen failure).

For taking truly representative core of the invasive tumor component see instructions in the Michelangelo ETNA Guidelines.

Investigations performed as part of the patient's routine care before she has consented to the study, may be used as study assessments (with the exception of the serum or urine pregnancy test in women of child-bearing potential) of and do not need to be repeated as long as they fall within 2 weeks prior to the date of signature of Informed Consent.

tumor core biopsy for central laboratory review can be shipped to the referral laboratory only after all necessary screening data have been entered in the e-CRF. To clarify how to proceed should the first sample turn out to be inadequate at the referral laboratory.
| Section 7 Paragraph 7.1 | […] bilateral mammography or computerized tomography (CT) or magnetic resonance imaging (MRI) […] | bilateral mammography or computerized tomography (CT) or magnetic resonance imaging (MRI) or ultrasound (US) | To allow the use of US in screening procedures |
| Section 7 Paragraph 7.1 | Serum or urine pregnancy test in women of child-bearing potential (a negative serum pregnancy test outside the 14-days window may be confirmed with a negative pregnancy test) | Serum or urine pregnancy test in women of child-bearing potential (a negative serum pregnancy test must be provided within 72 hours prior starting study drug treatment) | Changes in the safety profile of the study drug |
| Section 7 Paragraph 7.2.2 | Evaluations to be performed before each abraxane/paclitaxel administration | Evaluations to be performed before each abraxane/paclitaxel administration (allowed within 48 hours prior to the planned treatment administration) | To allow participating sites to perform reported examinations within 48 h prior to the planned administration |
| Section 7 Paragraph 7.2.3 | Evaluations to be performed before each treatment cycle | Evaluations to be performed before each treatment cycle (allowed within 48 hours prior to the planned treatment administration) | To allow participating sites to perform reported examinations within 48 h prior to the planned administration |
| Section 7 Paragraph 7.4 | For the purposes of the study, the following investigations must be summarized on the e_CRF once a year: Physical examination and assessment of possible tumor recurrence Hematological and biochemistry examinations Imaging tests to rule out the presence of distant metastases Bone nuclear imaging to rule out metastases is mandatory if alkaline phosphatase is > ULN or if patient has unexplained bone pain Liver imaging to rule out metastases is mandatory in patients with AST or alkaline phosphatase > ULN Cardiac examination: Electrocardiograph (ECG), Left ventricular ejection fraction (LVEF) by echography or multi-gated scintigraphic scan (MUGA scan) for the first 2 years | The following investigations are suggested yearly, investigators may wish to see their patients more frequently according to their routine practice: Physical examination and assessment of possible tumor recurrence Mammography (bilateral in case of conservative surgery) Treatment related AE assessment Hematological and biochemistry examinations Imaging tests to rule out the presence of distant metastases Bone nuclear imaging to rule out metastases is mandatory if alkaline phosphatase is > ULN or if patient has unexplained bone pain Liver imaging to rule out metastases is mandatory in patients with AST or alkaline phosphatase > ULN Cardiac examination: Electrocardiograph (ECG), Left ventricular ejection fraction (LVEF) by echography or multi-gated scintigraphic scan (MUGA scan) for the first 2 years | To clarify that the reported investigations are suggested but not mandatory To clarify that only a brief summary of them need to be reported in the e-CRF |
### Section 7 Paragraph 7.5.1

**Local recurrence**

Recurrent local tumor is defined as evidence of breast cancer in the ipsilateral breast or skin of the breast. Patients who develop clinical evidence of tumor recurrence in the ipsilateral breast must have a biopsy of the suspicious lesion to confirm the diagnosis. **Ipsilateral breast tumor recurrence (IBTR)**

An IBTR event is defined as recurrent tumor in either the ipsilateral breast parenchyma or skin of the breast occurring after conservative surgery. Patients who develop clinical evidence of tumor recurrence in the ipsilateral breast must have a biopsy of the suspicious lesion to confirm the diagnosis. **Ipsilateral breast tumor recurrence (IBTR)**

Acceptable: Positive cytology or histological biopsy.

**Local progression**

Local recurrence is defined as evidence of breast cancer (invasive of in situ) in the ipsilateral breast or skin of the breast after surgery. When local recurrence occurs in the ipsilateral breast parenchyma after conservative surgery it is termed 'ipsilateral breast tumor recurrence (IBTR)'. Patients who develop clinical evidence of tumor recurrence in the remainder of the ipsilateral breast must have a biopsy of the suspicious lesion to confirm the diagnosis. **Ipsilateral breast tumor recurrence (IBTR)**

Acceptable: Positive cytology or histological biopsy.

To clarify that disease progression while on primary therapy is included and to clarify that biopsy is mandatory for ipsilateral breast tumor recurrence.

### Section 7 Paragraph 7.5.2

**Other local recurrence**

Defined as recurrence in the skin of the chest wall (exclusive of the breast) or chest wall. **Other local recurrence/progression**

Defined as the appearance of breast cancer in the skin of the chest wall (exclusive of the breast) or chest wall after surgery (recurrence) or during primary chemotherapy (progression).

To better clarify other local recurrence.

### Section 7 Paragraph 7.5.4

**Regional recurrence**

Defined as the development of tumor in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes, as well as the soft tissue of the ipsilateral axilla, following surgery. **Regional recurrence/Regional progression**

Defined as increase in size or new appearance of the tumor in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes, as well as the soft tissue of the ipsilateral axilla.

To clarify that disease progression while on primary therapy is included and to better clarify regional disease.

### Section 8 Table 6

**Title**

Dose Reduction for Abraxane at the start of a new cycle

Dose Reduction for Abraxane

To clarify that dose reduction is valid for all administrations.

**Bottom note b**

b [...] If platelets have not recovered on day 35, discontinue treatment

b [...] If platelets and/or neutrophils have not recovered on day 35, discontinue treatment

To clarify that also neutrophils had to recover on day 35.
<table>
<thead>
<tr>
<th>Section</th>
<th>Paragraph 8.1.2</th>
<th>Absent</th>
<th>[…] at least 1 week (+/- 2 days)</th>
<th>To allow administering interval for sequential chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section</td>
<td>Paragraph 8.1.2</td>
<td>[…] WFI</td>
<td>[…] as per Summary of Product Characteristics</td>
<td>To be consistent with SmPC</td>
</tr>
<tr>
<td>Section</td>
<td>Table 9 Title</td>
<td>Dose Reduction for Paclitaxel at the start of a new cycle</td>
<td>Dose Reduction for Paclitaxel</td>
<td>To clarify that dose reduction is valid for all administrations</td>
</tr>
<tr>
<td>Section</td>
<td>Table 9 Bottom note b</td>
<td>b[…] If platelets have not recovered on day 35, discontinue treatment</td>
<td>b[…] If platelets and/or neutrophils have not recovered on day 35, discontinue treatment</td>
<td>To clarify that also neutrophils had to recover on day 35</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 8.1.3</td>
<td>AC will be started on day 21 after the last administration of abraxane or paclitaxel (toxicity permitting) according to the following doses and schedule</td>
<td>AC will be started between day 14 and day 21 after the last administration of abraxane or paclitaxel (toxicity permitting) according to the following doses and schedule</td>
<td>To allow administering sequential chemotherapy on day 15 after the last dose of either taxane</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 8.1.3</td>
<td>Absent</td>
<td>Other Dilution Procedures as per local standard are allowed</td>
<td>To be consistent with local standard</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 8.1.3</td>
<td>Absent</td>
<td>[…] every 21 days (+/- 2 days)</td>
<td>To allow administering interval for sequential chemotherapy</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 8.1.4</td>
<td>FEC will be started on day 21 after the last administration of abraxane or paclitaxel (toxicity permitting) according to the following doses and schedule</td>
<td>FEC will be started between day 14 and day 21 after the last administration of abraxane or paclitaxel (toxicity permitting) according to the following doses and schedule</td>
<td>To allow administering sequential chemotherapy on day 15 after the last dose of either taxane</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 8.1.4</td>
<td>Absent</td>
<td>Other Dilution Procedures as per local standard are allowed</td>
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<tr>
<td>Section</td>
<td>Paragraph 8.1.4</td>
<td>Absent</td>
<td>[…] every 21 days (+/- 2 days)</td>
<td>To allow administering interval for sequential chemotherapy</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 11.1</td>
<td>Formalin Fixed Paraffin Embedded (FFPE) tumor block at diagnostic biopsy and at definite surgery (mandatory)</td>
<td>Formalin Fixed Paraffin Embedded (FFPE) tumor block at diagnostic biopsy (mandatory) and at definite surgery (recommended)</td>
<td>To clarify that FFPE tumor block is mandatory only at diagnosis</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 11.1</td>
<td>After the first cycle of abraxane or paclitaxel (before the start of the second cycle)</td>
<td>After the first cycle of abraxane or paclitaxel (recommended before the start of the second cycle between day 21 and day 24)</td>
<td>To recommend timing for the biopsy</td>
</tr>
<tr>
<td>Section</td>
<td>Paragraph 11.1</td>
<td>Not present</td>
<td>Tissue collection at disease progression or recurrence (if any and subject to patient consent) For patients diagnosed with a local progression or recurrence or contralateral breast cancer, it is suggested the submission of an FFPE tumor block from the biopsy or surgical sample (preferred) that was used to diagnose progressive or</td>
<td>To allow collection of tumor tissue at disease progression or recurrence if any</td>
</tr>
</tbody>
</table>
| Section 11 Paragraph 11.3 | Baseline and after chemotherapy Whole Blood, Serum and Plasma Samples (subject to patient consent) 
At baseline (prior to the start of cycle 1) and after the chemotherapy treatment (before definite surgery), whole blood, serum and plasma samples (each prepared from 10 mL of peripheral blood) will be collected for biomarker assessment | **Collection of Whole Blood, Serum and Plasma Samples (subject to patient consent)** 
At baseline (prior to the start of cycle 1), Before the start of the second cycle between day 21 and day 24 and after the chemotherapy treatment (before definite surgery), **and at disease progression or recurrence (if any)** whole blood, serum and plasma samples (each prepared from 10 mL of peripheral blood) will be collected for biomarker assessment | To allow collection of blood samples at disease progression or recurrence (if any) |
| Section 11 Figure 3 | At the end of the 1st cycle of abraxane/paclitaxel (recommended):  
• One paraffin block for biomarker analysis to be sent to the to the study's central biological sample repository | **Before the start of the second cycle between day 21 and day 24 (recommended):**  
• One paraffin block for biomarker analysis to be sent to the to the study's central biological sample repository  
• Whole blood, serum and plasma samples (10 mL each) to be stored at the site and at defined timeline to be sent to the study’s central biological sample repository | To better clarify timing of tissue sample after the first cycle and to allow collection of blood sample |
| Section 11 Figure 3 | Not present | **At disease progression or recurrence (local or metastatic-IF ANY):**  
• One paraffin block or fine needle biopsy for biomarker analysis to be sent to the to the study's central biological sample repository  
• Whole blood, serum and plasma samples (10 mL each) to be stored at the site and at defined timeline to be sent to the study’s central biological sample repository | To allow collection of tumor tissue and blood samples at disease progression or recurrence, if any |
| Section 12.4.1 | Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age) of a female subject occurring while the subject is on protocol drug(s) delivered, or within 28 days of the subject’s last dose of protocol drug(s), are considered immediately reportable events. | Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age) of a female subject occurring while the subject is on protocol drug(s) delivered, or within 3 months of the subject’s last dose of protocol drug(s), are considered immediately reportable events. | Changes in the safety profile of the study drug. |
**Study synopsis ETNA / FM-12-B01**

<table>
<thead>
<tr>
<th>TITLE</th>
<th>Neoadjuvant chemotherapy with nab-paclitaxel in women with HER2-negative high-risk breast cancer ETNA (Evaluating Treatment with Neoadjuvant Abraxane)</th>
</tr>
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<tbody>
<tr>
<td>SPONSOR</td>
<td>Fondazione Michelangelo</td>
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<tr>
<td>PROTOCOL PHASE</td>
<td>Phase III</td>
</tr>
<tr>
<td>TRIAL CONDUCT</td>
<td>This multicentre trial will be conducted under the principal sponsorship and overall trial management of the Fondazione Michelangelo. The study will be done in collaboration with regional European and non-European cooperative groups. Within each country/region, day-to-day management including site feasibility, monitoring and serious adverse event management will be undertaken by a nominated local centre.</td>
</tr>
<tr>
<td>INDICATION</td>
<td>Patients with HER2-negative, not metastatic unilateral breast cancer who are at risk of disease recurrence and suitable for neoadjuvant chemotherapy</td>
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</table>
| OBJECTIVES | **Primary Objective**  
- To compare the rate of pathologic Complete Response (pCR, defined as ypT0-Tis, ypN0) for abraxane (Abraxane®, abraxane) vs paclitaxel  

**Secondary Objectives**  
- To compare the pCR rates in the two main subgroups of ER and/or PgR positive tumors and triple-negative tumors separately  
- To compare the rate of clinical overall response (cOR) after the first 4 cycles of abraxane vs paclitaxel  
- To compare the rate of cOR after the entire preoperative chemotherapy (i.e. before surgery) in the study arms of abraxane vs paclitaxel  
- To compare the Event Free Survival (EFS, i.e. disease progression while on primary therapy or disease recurrence after surgery) in the study arms of abraxane vs paclitaxel  
- To compare the Distant EFS (DEFS) in the study arms of abraxane vs paclitaxel  
- To compare the Local EFS (LEFS) in the two study arms of abraxane and paclitaxel  
- To compare the Regional EFS (REFS) in the two study arms of abraxane and paclitaxel  
- To compare the overall survival (OS) in the study arms of abraxane vs paclitaxel  
- To evaluate the tolerability of the treatment regimens in the different study arms  
- To conduct molecular and clinical analyses to assess the presence of predictive markers of benefit |
| STUDY DESIGN | This is an open-label, randomized phase III trial. Patients will be randomized to one of the 2 possible treatment arms in a 1:1 ratio (Arm A, abraxane/Arm B, paclitaxel):  
- Stratification variables will include |
### NUMBER OF PATIENTS

A planned total of approximately 632 randomized patients.

### ELIGIBILITY CRITERIA

#### Inclusion Criteria

Patients must meet ALL of the following criteria in order to be eligible for this study:

1. Female patients aged 18 years or older
2. Histologically confirmed invasive unilateral breast cancer
3. HER2-negative disease (defined as 0-1+ by immunohistochemistry or 2+ by immunohistochemistry without HER2 amplification by either FISH, CISH, or other amplification tests done locally)
4. Known hormone receptor status (estrogen receptor [ER], progesterone receptor [PgR]), tumor grade and, if institutional standard permits, known Ki67 value
5. Available paraffin-embedded tumor block taken at diagnostic biopsy for central confirmation of HER2 eligibility, hormone receptor status, Ki67 value and biomarker evaluation is mandatory
6. One of the following breast cancer stages:
   a. T2, T3, T4a-d disease, triple negative (HER2, ER, PgR) regardless of Ki67 value
   b. T2, T3, T4a-d disease, ER and/or PgR positive. If Ki67 can be performed at the site, local Ki67 value must be ≥ 14%. If Ki67 is not available at the site, the tumor grade must be assessed as grade 2 or 3
7. ECOG performance status 0 or 1
8. Written informed consent to participate in the trial (approved by the Institutional Review Board [IRB]/ Independent Ethics Committee [IEC]) obtained prior to any study specific screening procedures
9. Willing and able to comply with the protocol

#### Exclusion Criteria

Patients meeting any ONE of the following criteria are not eligible for this study:

1. Synchronous contralateral breast cancer or presence of metastatic disease (M1). Exception: contralateral in situ ductal cancer
2. Surgical axillary staging procedure prior to study entry. Exceptions: 1) FNA of an axillary node is permitted for any patient, and 2) although not recommended, a pre-neoadjuvant therapy sentinel lymph node biopsy for patients with clinically negative axillary nodes is permitted
3. Pregnant or lactating women. Documentation of a negative pregnancy test must be available for premenopausal women with intact reproductive organs and for women less than one year after the last menstrual cycle.

4. Women with childbearing potential unless (1) surgically sterile or (2) using adequate measures of contraception, for example abstinence, an intra-uterine device, or double barrier method of contraception.

5. Treatment including radiation therapy, chemotherapy, biotheraphy, and/or hormonal therapy for the currently diagnosed breast cancer prior to study entry.

6. Previous investigational treatment for any condition within 4 weeks of randomization date.

7. Patients on therapy with a strong CYP3A4 inhibitor and on therapy with Warfarin (Coumadin).

8. Previous or concomitant malignancy of any other type that could affect compliance with the protocol or interpretation of results. Patients with curatively treated basal cell carcinoma of the skin or in situ cervix cancer are generally eligible.

9. Pre-existing motor or sensory neuropathy of grade > 1 for any reason.

10. Patients with a history of hypersensitivity due to drugs containing polyoxyethylene castor oil (Cremophor EL) (e.g., ciclosporin), or hardened castor oil (e.g., vitamin preparations for injection, etc.)

11. Other serious illness or medical condition including: history of documented congestive cardiac failure; angina pectoris requiring anti-anginal medication; evidence of transmural infarction on ECG; poorly controlled hypertension (e.g. systolic >180 mm Hg or diastolic >100 mm Hg; however, patients with hypertension which is well controlled on medication are eligible); clinically significant valvular heart disease; high-risk uncontrolled arrythmias.

12. Patients with a history of uncontrolled seizures, central nervous system disorders or psychiatric disability judged by the investigator to be clinically significant and precluding informed consent or adversely affecting compliance with study drugs.

13. Serious uncontrolled infections (bacterial or viral) or poorly controlled diabetes mellitus.

14. Any of the following abnormal baseline hematological values:
   a. Absolute Neutrophil Count (ANC) < 1.5 \times 10^9/L
   b. Platelet count < 100 \times 10^9/L
   c. Hemoglobin (Hb) < 10 g/dL

15. Any of the following abnormal baseline laboratory tests:
   a. Serum total bilirubin > 1.5 \times ULN (upper limit of normal) (except for patients with clearly documented Gilbert’s syndrome).
   b. Alanine transaminase (ALT) or aspartate transaminase (AST) > 1.25 \times ULN
   c. Alkaline phosphatase > 2.5 \times ULN
   d. Serum creatinine > 1.5 \times ULN

16. Baseline left ventricular ejection fraction (LVEF) < 50% by echocardiography or multi-gated scintigraphic scan (MUGA).

**LENGTH OF STUDY**

The primary endpoint will be evaluated at the time of surgery. Assuming an escalating accrual rate in the first 6 months of the trial and a uniform accrual thereafter, the total enrolment of 632 patients will results in
completion of enrolment in approximately 30 months. The primary analysis will be performed approximately 40 months after the randomization of the first patient that is when all the 632 patients required by sample size specifications have completed their courses of treatment and reached the surgery phase. The Clinical Study Report will be written when the evaluation of the primary endpoint pCR takes place. The first analysis of EFS will take place 5 years after the randomization of the first patient and data will be reported in the CRS as a first addendum; At this early stage it’s very likely to have many censored values for this endpoint, i.e. most of the patients will not have experienced the event of interest. In order to get more mature data, each randomized patient will be followed until the occurrence of the event up to 10 years after randomization. When the time frame is completed the final analysis of EFS will be performed, jointly with the analysis of overall survival, and reported as a final addendum.

**TREATMENT PLAN**

All drug will be delivered intravenously

**Arm A**

*Abraxane* at the dosage of 125 mg/m² over 30 minutes given week 1, 2 and 3 followed by 1 week rest to be repeated for 4 cycles

*followed by*

*AC* or *EC* (adriamycin or epirubicin and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

*or*

*FEC* (fluorouracil, epirubicin, and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

**Arm B**

*Paclitaxel* at the dosage of 90 mg/m² diluted in 250 mL as per Summary of Product Characteristics (SmPC) over 1 hour given week 1, 2 and 3 followed by 1 week rest to be repeated for 4 cycles

*followed by*

*AC* or *EC* (adriamycin or epirubicin and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

*or*

*FEC* (fluorouracil, epirubicin, and cyclophosphamide) on day 1 every three weeks for 4 cycles

After completion of the neoadjuvant and surgical treatment patients will receive irradiation as per accepted international and local guidelines. Patients subjected to breast sparing surgery must receive breast irradiation. Partial breast irradiation techniques utilizing brachytherapy are not permitted.

Patients with hormone receptor positive disease (ER and/or PgR-positive) will also receive hormone therapy. The investigator’s choice of hormone therapy for each individual patient must be decided according to local guidelines and documented in the e-CRF.
### ASSESSMENTS OF

- **Efficacy**

  **Primary efficacy variable**
  The primary endpoint is pathological complete response (pCR) defined as absence of invasive disease in breast and nodes (ypT0/ypTis, ypN0).

- **Safety**

  Patients will be assessed for adverse events by clinical examination, questioning for symptoms of toxicity, laboratory assessments, vital signs, ECG and LVEF.

  Neurological toxicity and other toxicities will be assessed throughout the study according the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Event (CTCAE) Version 4.0.

### PROCEDURES (Summary)

Eligible and consenting patients will be randomized and will undergo 4 cycle of taxane neoadjuvant chemotherapy followed by 4 cycles of an anthracycline-containing regimen chosen by the Investigator.

Neoadjuvant chemotherapy will be followed by definite surgery and irradiation as per international and local guidelines.

During neoadjuvant chemotherapy patients will be assessed for safety and efficacy as detailed in the protocol.

After definite surgery patients will be followed for approximately 10 years according to local procedures.

### STATISTICAL ANALYSIS

The primary endpoint is to compare the rate of pathologic Complete Response (pCR) between abraxane and paclitaxel. Treatment contrast is expressed in terms of relative measure of effect, i.e. an odds ratio (OR) equal to 1.7, corresponding to an absolute difference of 10% when the pCR for paclitaxel is 20%, setting a target pCR for abraxane to 30%.

The derived OR is then used to employ a two sided Cochran-Mantel-Hanszel design, stratifying the comparison between the 2 arms by breast cancer phenotype (triple negative, others).

Assuming to screen 40% patients triple negative and 60% patients with other tumors, with an estimated response rate to paclitaxel of 32% and 15%, respectively, 632 patients (316 per arm) are required to reject the OR set by the null hypothesis of 1 when the OR is actually 1.7, with power=80% and the false positive rate=5%.

The expected overall pathological complete response rate in breast and axilla (pCR) for HER2- patients with operable and locally advanced breast cancer, that is 20%, is taken from results of the GeparTrio study (Huober et al., 2010) where patients were essentially treated with a docetaxel containing regimen. In the GeparTrio trial, in a sample of about 1000 HER2- patients, a pCR at surgery was recorded for 20% of them. The updated analysis presented at the San Antonio Breast Cancer Conference (abstract n° S3-2, 2011) reported a pCR for 133 patients out of 362 triple negative patients (36.7%) and for 39 patients out of 211 luminal B patients (18.5%).

Estimates of paclitaxel effect in the 2 study sub-populations (triple negative vs other) are based on the NOAH study in locally advanced breast cancers (Gianni et al, Lancet 2010). In the NOAH study, in the limited parallel group of HER2-negative tumors 11 patients out of 35 triple
negative patients (31%) and 2 patients out of 16 in the other tumors (12.5%) attained a pCR. Because of the statistical considerations set forth in Section 9. (Statistical considerations and analysis plan), enrolment to either of the two tumor subtypes will be discontinued after reaching the estimated percentage and will be continued only on the other subtype to reach a total of 632 patients.

Sample size computations were made using Pass 2008 software.
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1. GLOSSARY OF ABBREVIATIONS

AE   Adverse event
AC   Adriamycin (doxorubicin), Cyclophosphamide
ALND Axillary Lymph Node Dissection
ALP  Alkaline Phosphatase
ALT  Alanine Aminotransferase
ANC  Absolute Neutrophil Count
AJCC American Joint Committee on Cancer
AST  Aspartate Amino-Transferase
BSA  Body Surface Area
CFISH Comet Fluorescent in Situ Hybridization
CI   Confidence Interval
CISH Chromogenic in Situ Hybridization
CRF  Case Report Form
eCRF Electronic Case Report Form
cOR  Clinical Overall Response
CT   Computerized Tomography
CTC AE Common Terminology Criteria for Adverse Events
D5W  Dextrose 5% in water
EFS  Disease Free Survival
DEFS Distant Disease Free Survival
DMC  Data Monitoring Committee
DNA  Deoxyribonucleic Acid
EC   Epirubicin, Cyclophosphamide
ECG  Electrocardiography
ECOG Eastern Cooperative Oncology Group
EDC  Electronic Data Capture
EDTA Ethylenediaminetetra-acetic acid
ER   Estrogen Receptor
FFPE Formalin Fixed Paraffin Embedded
FAC  Fluorouracil, Adriamycin (doxorubicin), Cyclophosphamide
FEC  Fluorouracil, Epirubicin, Cyclophosphamide
FISH Fluorescent in situ hybridization
FNA  Fine needle aspiration
HER2 Human Epidermal Growth Factor Receptor 2 Protein
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RRFS</td>
<td>Regional Recurrence Free Survival</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SAP</td>
<td>Statistical Analysis Plan</td>
</tr>
<tr>
<td>SGOT</td>
<td>Serum Glutamic Oxaloacetic Transaminase</td>
</tr>
<tr>
<td>SGPT</td>
<td>Serum Glutamic-Pyruvic Transaminase</td>
</tr>
<tr>
<td>SOC</td>
<td>System Organ Class</td>
</tr>
<tr>
<td>TEAE</td>
<td>Treatment Emergent Adverse Events</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to Progression</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
</tr>
<tr>
<td>UPN</td>
<td>Unique Patient number</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Count</td>
</tr>
<tr>
<td>WFI</td>
<td>Water For Injection</td>
</tr>
</tbody>
</table>
2. BACKGROUND AND RATIONALE

2.1 Introduction

In spite of major progresses in the combined use of surgery, irradiation and adjuvant systemic therapy, a significant percentage of patients with diagnosis of early breast cancer still relapse and die, usually due to distant disease recurrence. Therefore the goal of optimizing current therapies and integrating new treatments into adjuvant therapy remain areas of active clinical research.

Several large trials have established the efficacy of adjuvant trastuzumab in patients with HER2-positive early breast cancer while there are currently several areas of uncertainty in the adjuvant treatment of patients with HER2-negative early breast cancer. One issue is the selection of patients with estrogen receptor and/or progesterone receptor [ER/PgR]-positive disease for adjuvant chemotherapy. Patients with such tumor characteristics benefit from adjuvant hormone therapy. The decision whether to also offer adjuvant chemotherapy is currently based on conventional clinical and pathological risk factors. However, such criteria predict disease recurrence and progression imperfectly. As a consequence some or even many patients are treated with chemotherapy unnecessarily, whereas others who may benefit from chemotherapy do not receive it.

Gene expression is recognized as an important indicator of tumor behavior and gene expression profiling has emerged as a better predictor of clinical outcome in hormone receptor positive patients treated with adjuvant tamoxifen. A Panel of international experts has proposed a classification of patients for therapeutic purposes based on the recognition of molecular subtypes. For practical purposes these subtypes may be approximated using clinical-pathological rather than gene array expression criteria. The Panel supported the determination of ER, PgR, HER2 and Ki-67 as useful for defining Luminal A (ER/PgR positive, HER2 negative, Ki-67 low), Luminal B HER2 negative (ER/PgR positive, Ki-67 high), Luminal B HER2 positive (ER/PgR positive, any Ki-67), HER2 positive non luminal (ER and PgR negative, any Ki-67), Triple negative (HER2 negative, ER and PgR negative, any Ki-67).

2.2 Rationale for conducting a Phase III neoadjuvant study

Neoadjuvant chemotherapy is an established approach for managing patients with large early stage breast cancer and with locally advanced disease, and provides an opportunity for collection of specimens for correlative science studies to identify predictive markers for response to specific agents.

The rationale for evaluating preoperative chemotherapy in patients with non-metastatic breast cancer was provided by hypotheses formulated from findings obtained in laboratory investigations and from results of preoperative chemotherapy for early stage and locally advanced cancers. The presence of clinically and pathologically assessable disease allows for assessing the response to the administered neoadjuvant regimen and provides a unique opportunity to evaluate pretreatment specimens for predictive markers.

To potentially avoid overtreatment in patients with hormonal receptor positive tumors, in our study we will use the above mentioned classification to select HER2 negative high risk tumors to be treated, namely Luminal B and triple negative categories.

Due to the success of available therapies, the conduct of adjuvant studies requires ever larger numbers of patients and long periods of follow up to assess the value of new
therapies. In recent years the neoadjuvant approach is gaining increasing consideration as a tool for the rapid scrutiny and ranking of new and established therapies, especially since the use and application in groups of patients selected for homogeneous molecular characteristics.

The overall concept of the approach and the overall experimental design is in two phases as illustrated in the following scheme:

![Figure 1: Overall Design](image)

In the present study the neoadjuvant approach will be used to compare the complete pathological response rate of two regimens: a) weekly abraxane (Abraxane®, abraxane) followed by AC or EC or FEC before surgery; b) weekly paclitaxel followed by AC or EC or FEC before surgery.

In our study we will use hormonal receptor status and Ki67 values to select HER2 negative tumors at high risk of disease relapse, namely Luminal B and triple negative categories.

Also, several IHC and molecular assays will be performed before and during the period of chemotherapy administration and at surgery with the goal of defining a marker of efficacy to be later validated in a larger adjuvant setting.

### 2.2.1 Justification for the use of pathologic complete response as primary endpoint

Randomized neoadjuvant trials suggest that a pathologic complete response (pCR) may predict disease-free survival (Table 1) or overall survival among patients with breast cancer who are treated with preoperative systemic therapy. If higher pCR rates obtained with more effective regimens continue to predict for improved outcome, then pCR could be used as an intermediate endpoint in testing new chemotherapy regimens as well as newer targeted therapies. For example, in a neoadjuvant trial of chemotherapy with or without trastuzumab in locally advanced breast cancer, the group that received trastuzumab had a near doubling of the
pCR rate (38% vs 19%, p = 0.001) that translated into a statistically significant 3-year disease-free survival rate of 71% vs 50% (p = 0.013).

<table>
<thead>
<tr>
<th>First Author</th>
<th>Treatment</th>
<th>pCR (%)</th>
<th>DFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hutcheon AW</td>
<td>CVAP vs CVAP→D</td>
<td>16 vs 34</td>
<td>3-yr 77 vs 90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p = 0.035)</td>
<td>(p = 0.03)</td>
</tr>
<tr>
<td>Untch M</td>
<td>ET vs E→T</td>
<td>10 vs 18</td>
<td>5-yr 50 vs 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p = 0.008)</td>
<td>(p = 0.011)</td>
</tr>
<tr>
<td>Gianni L</td>
<td>AT→T→CMF vs AT→T→CMF+H</td>
<td>19 vs 38</td>
<td>3-yr 56 vs 71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p = 0.001)</td>
<td>(p = 0.013)</td>
</tr>
<tr>
<td>Buzdar AU</td>
<td>T→FEC vs T→FEC+H</td>
<td>26 vs 65</td>
<td>3-yr 85 vs 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p = 0.016)</td>
<td>(p = 0.041)</td>
</tr>
<tr>
<td>Bear HD</td>
<td>AC→S (a) vs AC→S (b)→D vs AC→D (c)→S</td>
<td>13 vs 14.5 vs 26</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p &lt; 0.001 a+b vs c)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Disease-free survival (DFS) in selected neoadjuvant chemotherapy studies

2.3 Phase III trials evaluating the addition of taxanes to anthracycline-based neoadjuvant chemotherapy regimens

Results from initial randomized trials of neoadjuvant chemotherapy strengthened the biologic and clinical rationale for continuing to evaluate its role in patients with breast cancer. Since response to neoadjuvant chemotherapy correlated with patient outcome, it was hypothesized that more active neoadjuvant chemotherapy regimens should increase the pCR rates and improve disease-free and overall survival. The demonstration of significant antitumor activity of taxanes in advanced breast cancer provided the opportunity to test these hypotheses in the neoadjuvant setting. Several randomized trials were designed to evaluate neoadjuvant chemotherapy regimens that employed anthracyclines and taxanes. The rates of pCR have essentially been doubled with the addition of a sequential taxane to anthracycline/cyclophosphamide combinations.

The optimal chemotherapy regimen and sequence for patients with early breast cancer has not been defined yet. Paclitaxel by weekly schedule of administration is currently used as one of the most effective chemotherapies after administration of an anthracycline-containing regimen (AC, EC, FEC, FAC). Reversal of the sequence of the regimens does not appear to reduce the activity. A neoadjuvant trial reported by MD Anderson evaluated 2 schedules of paclitaxel followed by 4 cycles of fluorouracil/doxorubicin/cyclophosphamide (FAC) in standard doses every three weeks. A total of 258 patients were randomized to receive paclitaxel either weekly (for a total of 12 doses) or every three weeks (4 cycles), followed by FAC. Patients receiving weekly paclitaxel followed by FAC had a pCR rate of 28% which is similar to the pCR rate reported...
in regimens starting with anthracyclines followed by taxanes.\textsuperscript{15} A multicenter neoadjuvant study randomly compared 4 cycles of an anthracycline based chemotherapy followed by 4 cycles of docetaxel versus the inverse sequence. The rate of pCR was similar in the two treatment groups (28\% vs 29\%) and no difference was observed in the median disease-free survival.\textsuperscript{16}

2.4 Rationale for using albumin bound (AB) nab-paclitaxel

The two most widely used taxanes are paclitaxel and docetaxel. They are highly hydrophobic and need solvents for intravenous administration. Being biologically and pharmacologically active, these solvents are associated with several major side effects and impair tumor penetration, limiting the clinical effectiveness of solvent-based taxanes.

To address the limitations of solvent-based taxanes and to improve the therapeutic index, various solvent-free formulations and delivery systems were investigated with limited success. The first successful attempt to formulate a solvent-free taxane was the development of albumin bound-paclitaxel. The nano-particle protein platform utilizes the natural properties of albumin to increase drug delivery to the tumor and eliminates the need for solvents.

\textit{Nab}-paclitaxel (ABI-007, Abraxane\textregistered, abraxane) is a novel formulation of solvent free 130-nanometer human albumin-bound paclitaxel, an innovative formulation of paclitaxel that allows for the safe administration of rapid infusions without need for premedication. At the same time, the formulation allows for preferential delivery of the drug to the tumor by exploiting \textit{ad hoc} mechanisms of transport. In the setting of metastatic breast cancer abraxane has been associated with improved efficacy with respect to the conventional taxanes paclitaxel and docetaxel. It is therefore appealing to test \textit{nab}-paclitaxel in the subgroups of women with early high risk breast cancer characterized by absence of HER2 expression.

2.4.1 Phase I clinical experience with abraxane single agent

Three different dose schedules of abraxane have been evaluated in a Phase I study by Ibrahim et al.\textsuperscript{17} 19 patients with advanced solid tumors received abraxane as a 30 minute infusion every 3 weeks without premedication using doses from 135 to 375 mg/m\textsuperscript{2}. No infusion related acute hypersensitivity reactions were documented. At the highest dose level, dose-limiting toxicity occurred in 3 of 6 patients and consisted of sensory neuropathy (3 patients), stomatitis (2) patients and superficial keratopathy (2 patients). The MTD was determined to be 300 mg/m\textsuperscript{2}. In another phase I study by Nyman et al.\textsuperscript{18} 39 patients received abraxane without premedication at a dose levels from 80 to 200 mg/m\textsuperscript{2} over a 30-minute infusion once a week for 3 weeks in each monthly cycle. The MTD dose was 150 mg/m\textsuperscript{2} in ‘lightly’ pretreated patients and 100 mg/m\textsuperscript{2} in ‘heavily’ pretreated patients. Dose limiting toxicities were grade 3 peripheral neuropathy and grade 4 neutropenia, respectively.

2.4.2 Phase II studies of single agent nab-paclitaxel in advanced breast cancer (Table 2)

Ibrahim et al.\textsuperscript{19} delivered 300 mg/m\textsuperscript{2} of abraxane without premedication every 3 weeks to 63 women with measurable advanced breast cancer, 48 of whom had received prior chemotherapy for advanced disease. The median number of treatments was 6 cycles. Overall response rate (ORR) was 48\%, median time to progression (TTP) was 26.6 weeks and the median overall survival (OS) was 63.6 weeks. No severe hypersensitivity reactions were reported.
Blum et al.\textsuperscript{20} delivered 100 mg/m\textsuperscript{2} or 125 mg/m\textsuperscript{2} of abraxane given once a week for 3 weeks in each monthly cycle in patients with advanced breast cancer who had failed conventional taxane treatment. Response rates were 14\% and 16\% in the two cohorts respectively and also progression-free survival (PFS) and OS were similar. No severe hypersensitivity reactions were reported.

Gradishar et al.\textsuperscript{21} conducted a randomized phase II study comparing three different schedules of nab-paclitaxel and docetaxel (Table 2) given as first line treatment. Investigator’s assessed ORRs and PFS were significantly higher for the two weekly schedules of abraxane compared to docetaxel. Peripheral neuropathy was similar in all treatment groups but resolved more rapidly after treatment withdrawal with abraxane.

<table>
<thead>
<tr>
<th>First Author</th>
<th># patients</th>
<th>ORR</th>
<th>Median PFS in months</th>
<th>Median OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibrahim NK\textsuperscript{19}</td>
<td>63</td>
<td>48 % (total series) 64 % (first-line treatment)</td>
<td>6.2</td>
<td>14.6</td>
</tr>
<tr>
<td>Blum JL\textsuperscript{20}</td>
<td>181</td>
<td>14 (100 mg/m\textsuperscript{2}) 16 (125 mg/m\textsuperscript{2})</td>
<td>3 3.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Gradishar WJ\textsuperscript{21}</td>
<td>300</td>
<td>37 (300 mg/m\textsuperscript{2} q 3 wk) 45 (100 mg/m\textsuperscript{2}) 49 (150 mg/m\textsuperscript{2})</td>
<td>11 12.8 12.9</td>
<td>27.7 22.2 33.8</td>
</tr>
</tbody>
</table>

*delivered once a week for 3 weeks in each monthly cycle*

### 2.4.3 Phase II studies with regimens including nab-paclitaxel in Locally advanced breast cancer

A variety of combination regimens including abraxane have been or are being tested as either first or second line treatment in different patient subsets presenting with locally advanced or metastatic breast cancer followed by FEC as in the proposed trial. It is worth mentioning the phase II study by Robidoux et al.\textsuperscript{22} in locally advanced breast cancer. Sixty-five patients received 12 weekly cycles of abraxane at the dose of 100 mg/m\textsuperscript{2} followed by 4 cycles of FEC. Patients presenting with HER2-positive disease could receive concomitant trastuzumab starting from the first dose of the taxane. The overall pCR rate in the breast was documented in 29\% of patients (Table 3). The two-year PFS and OS were 81\% and 95\%, respectively. The most frequent adverse events during nab-paclitaxel treatment were fatigues (grade 2: 26\%, grade 3: 6\%) and peripheral neuropathy (grade 2: 11\%, grade 3: 5\%).
### Table 3: Phase II study of neoadjuvant nab-paclitaxel followed by FEC in locally advanced breast cancer

* could receive concomitant trastuzumab, HR: hormone receptor status

<table>
<thead>
<tr>
<th>Tumor category</th>
<th>Breast pCR</th>
<th>2-yr PFS</th>
<th>2-yr OS</th>
<th>Fatigue</th>
<th>Sensory neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total series</td>
<td>19/65 (29%)</td>
<td>81%</td>
<td>95%</td>
<td>G2: 37%</td>
<td>G2: 5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G3: 8%</td>
<td>G3: 0</td>
</tr>
<tr>
<td>HER2 neg, HR pos</td>
<td>3/28 (11%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 neg, HR neg</td>
<td>5/18 (28%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 pos*, HR pos</td>
<td>4/9 (44%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HER2 pos*, HR neg</td>
<td>7/10 (70%)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

2.4.4 Phase III studies with regimens including nab-paclitaxel in metastatic breast cancer (Table 4)

Based on the favorable data from phase I and phase II studies, efficacy and safety of nab-paclitaxel versus conventional paclitaxel in phase III randomized trials.

In the study reported by Gradishar et al., 454 patients were randomized to receive either nab-paclitaxel at the dose of 260 mg/m² without premedication or 175 mg/m² of conventional paclitaxel with corticosteroid and antihistamine premedication. ORR and TTP were significantly superior after abraxane than paclitaxel. About half of patients in each group received at least 6 treatment cycles. No severe hypersensitivity reactions occurred in the abraxane treatment group. Compared to the conventional paclitaxel, the incidence of grade 4 neutropenia was lower in the abraxane group (9% vs 22%), but the incidence of grade 3 sensory neuropathy was higher (10% vs 2%).

In another phase III trial, 2410 Chinese patients were randomly allocated to receive either 260 mg/2 of abraxane or 175 mg/m² of conventional paclitaxel. Similarly to the above study, ORR, TTP and PFS were significantly superior after abraxane.

The results from the CALGB 40502/NCCTG N063H randomly comparing weekly paclitaxel (90 mg/m²) vs weekly abraxane 150 mg/m² vs ixabepilone (16 mg/m²) with or without bevacizumab were recently reported. The ixabepilone arm was prematurely closed at the first interim analysis because the futility boundary for comparison with paclitaxel was crossed. The study was closed at the second interim analysis when the futility boundary for comparison of abraxane vs paclitaxel was crossed. Median PFS was 10.4 vs 9.6 vs 7.6 mos for paclitaxel, abraxane and ixabepilone, respectively. The authors concluded that both abraxane and ixabepilone are unlikely to be superior to conventional paclitaxel when combined with bevacizumab. Of note, approximately 45% of patients in the abraxane group discontinued treatment because of adverse events (mainly hematological toxicity of grade ≥ 3 and sensory neuropathy of grade ≥ 2).
Table 4: Phase III studies with regimens including nab-paclitaxel

* Once a week for 3 weeks in each monthly cycle and combined with bevacizumab; NR: not reported; HR: hazard ratio

3. STUDY DESIGN

This is an open-label, randomized phase III trial (Fig. 1).
Patients will be randomized to one of the 2 possible treatment arms in a 1:1 ratio (Arm A, abraxane/Arm B, paclitaxel):

Arm A

Abraxane at the dosage of 125 mg/m² over 30 minutes
given week 1, 2 and 3 followed by 1 week rest to be repeated for 4 cycles

followed by

AC or EC (doxorubicin or epirubicin and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

or

FEC (fluorouracil, epirubicin, and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

Arm B

Paclitaxel at the dosage of 90 mg/m² diluted in 250 mL as per Summary of Product Characteristics (SmPC) over 1 hour
given week 1, 2 and 3 followed by 1 week rest to be repeated for 4 cycles

followed by

AC or EC (adriamycin or epirubicin and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

or

FEC (fluorouracil, epirubicin, and cyclophosphamide) on day 1 every three weeks for 4 cycles
No neoadjuvant endocrine therapy is allowed in patients with hormone receptor positive disease (ER and/or PgR-positive).

The stratification variables will be:
  a) Cooperative Research Group
  b) Disease stage [operable (tumor stage:T2N0-1; T3N0) and locally advanced (T3N1; T4 any N; any T N2-3)]
  c) Tumor subtype [luminal B intermediate (HER2 negative, ER and/or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER and/or PGR positive, Ki67 >20%) vs triple negative tumors (HER2 negative, ER negative and PGR negative, Ki67 any value)]
Patients with HER2-negative, invasive unilateral breast cancer who are at risk of disease recurrence

1st Core

Randomize 1:1

Arm A
Abraxane week 1, 2 and 3 followed by 1 week rest for 4 cycles
followed by
AC (EC) or FEC for 4 cycles

Arm B
Paclitaxel week 1, 2 and 3 followed by 1 week rest for 4 cycles
followed by
AC (EC) or FEC for 4 cycles

2nd Core Biopsy before the start of the second cycle of ABX/PTX between day 21 and day 24

Surgery

Surgical Specimen

Follow-Up

Core Biopsy or a fine needle aspiration (FNA) at disease progression or recurrence (local or metastatic) if any

Figure 2: Study Design Flow-Chart
4. OBJECTIVES

4.1 Primary Objective

- To compare the rate of pathologic Complete Response (pCR, defined as ypT0-Tis, ypN0) for abraxane vs paclitaxel

4.2 Secondary Objectives

- To compare the pCR rates in the two main subgroups of ER and/or PgR positive tumors and triple-negative tumors separately
- To compare the rate of clinical overall response (cOR) after the first 4 cycles of abraxane vs paclitaxel
- To compare the rate of cOR after the entire preoperative chemotherapy (i.e. before surgery) in the study arms of abraxane vs paclitaxel
- To compare the Event Free Survival (EFS, i.e. disease progression while on primary therapy or disease recurrence after surgery) in the study arms of abraxane vs paclitaxel
- To compare the Distant EFS (DEFS) in the study arms of abraxane vs paclitaxel
- To compare the Local EFS (LEFS) in the two study arms of abraxane and paclitaxel
- To compare the Regional EFS (REFS) in the two study arms of abraxane and paclitaxel
- To compare the overall survival (OS) in the study arms of abraxane vs paclitaxel
- To evaluate the tolerability of the treatment regimens in the different study arms
- To conduct molecular and clinical analyses to assess the presence of predictive markers of benefit

4.3 Number of patients/assignment to treatment group

632 patients are required for the study. Patients will be randomized to one of the 2 possible treatment arms in a 1:1: ratio (Arm A/Arm B) resulting in approximately 316 patients in the experimental (with abraxane) arm and 316 patients in the control arm (with paclitaxel). Based on Fondazione Michelangelo database on breast cancer patients, we estimate that approximately 60% of the tumor subtypes will be luminal B and 40% will be triple negative. Because of the statistical considerations set forth in Section 9. (Statistical considerations and analysis plan), enrolment to either of the two tumor subtypes will be discontinued after reaching the estimated percentage and will be continued only on the other subtype to reach a total of 632 patients.

The treatment assigned will be based on a stratified randomization procedure using Cooperative Research Group; Disease stage [operative (tumor stage: T2N0-1; T3N0) and locally advanced (T3N1; T4 any N, any T N2-3)]; Tumor subtype [luminal B intermediate (HER2 negative, ER and/or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER and/or PGR positive, Ki67 >20%) vs triple negative tumors (HER2 negative, ER negative and PgR negative, Ki67 any value)].

4.4 End of study

The primary endpoint will be evaluated at the time of surgery; assuming that 2 to 4 weeks will elapse between the end of AC/EC/FEC regimen and the date of surgery, for each patient approximately 8 months since randomization are needed to meet the proper timing for primary analysis. Accounting for 30 months of accrual period, the primary endpoint will be analyzed ~ 40 months after the randomization of the first patient. At this stage the final study report will be prepared and released, including the results and discussion of all efficacy and...
safety analyses foreseen in the protocol and detailed in the statistical analysis plan of the study. In order to obtain more mature data for the secondary endpoints of DFS, DDFS, LRFS, RRFS and OS, the study duration will be extended up to 10 years after the randomization of the last participant into the study. These analyses will be reported separately from the final study report through an addendum.

5. STUDY POPULATION

5.1 Overview
Patients with HER2-negative not metastatic unilateral breast cancer who are at risk of disease recurrence and suitable for neoadjuvant chemotherapy.

5.2 Inclusion Criteria
Patients must meet ALL of the following criteria in order to be eligible for this study:

1. Female patients aged 18 years or older
2. Histologically confirmed invasive unilateral breast cancer
3. HER2-negative disease (defined as 0-1+ by immunohistochemistry or 2+ by immunohistochemistry without HER2 amplification by either FISH, CISH, or other amplification tests done locally)
4. Known hormone receptor status (estrogen receptor [ER], progesterone receptor [PgR]), tumor grade and, if institutional standard permits, known Ki67 value
5. Available paraffin-embedded tumor block taken at diagnostic biopsy for central confirmation of HER2 eligibility, hormone receptor status, Ki67 value and biomarker evaluation is mandatory
6. One of the following breast cancer stages:
   a. T2, T3, T4a-d disease, triple negative (HER2, ER, PgR) regardless of Ki67 value
   b. T2, T3, T4a-d disease, ER and/or PgR positive. If Ki67 can be performed at the site, local Ki67 value must be ≥ 14%. If Ki67 is not available at the site, the tumor grade must be assessed as grade 2 or 3
7. ECOG performance status 0 or 1
8. Written informed consent to participate in the trial (approved by the Institutional Review Board [IRB]/ Independent Ethics Committee [IEC]) obtained prior to any study specific screening procedures
9. Willing and able to comply with the protocol

5.3 Exclusion Criteria
Patients meeting any ONE of the following criteria are not eligible for this study:

1. Contralateral breast cancer or presence of metastatic disease (M1). Exception. Contralateral in situ ductal cancer
2. Surgical axillary staging procedure prior to study entry. Exceptions: 1) FNA of an axillary node is permitted for any patient, and 2) although not recommended, a pre-neoadjuvant therapy sentinel lymph node biopsy for patients with clinically negative axillary nodes is permitted
3. Pregnant or lactating women. Documentation of a negative pregnancy test must be available for premenopausal women with intact reproductive organs and for women less than one year after the last menstrual cycle
4. Women with childbearing potential unless (1) surgically sterile or (2) using adequate measures of contraception, for example abstinence, an intra-uterine device, or double barrier method of contraception.

5. Treatment including radiation therapy, chemotherapy, biotherapy, and/or hormonal therapy for the currently diagnosed breast cancer prior to study entry.

6. Previous investigational treatment for any condition within 4 weeks of randomization date.

7. Patients on therapy with a strong CYP3A4 inhibitor, and on therapy with warfarin (Coumadin).

8. Previous or concomitant malignancy of any other type that could affect compliance with the protocol or interpretation of results. Patients with curatively treated basal cell carcinoma of the skin or in situ cervix cancer are generally eligible.

9. Pre-existing motor or sensory neuropathy of grade > 1 for any reason.

10. Patients with a history of hypersensitivity due to drugs containing polyoxyethylene castor oil (Cremophor EL) (e.g., ciclosporin), or hardened castor oil (e.g., vitamin preparations for injection, etc.).

11. Other serious illness or medical condition including: history of documented congestive cardiac failure; angina pectoris requiring anti-anginal medication; evidence of transmural infarction on ECG; poorly controlled hypertension (e.g., systolic >180 mm Hg or diastolic >100 mm Hg; however, patients with hypertension which is well controlled on medication are eligible); clinically significant valvular heart disease; high-risk uncontrolled arrhythmias.

12. Patients with a history of uncontrolled seizures, central nervous system disorders or psychiatric disability judged by the investigator to be clinically significant and precluding informed consent or adversely affecting compliance with study drugs.

13. Serious uncontrolled infections (bacterial or viral) or poorly controlled diabetes mellitus.

14. Any of the following abnormal baseline hematological values:
   a. Absolute Neutrophil Count (ANC) < 1.5 \( \times 10^9 \) /L
   b. Platelet count < 100 \( \times 10^9 \) /L
   c. Hemoglobin (Hb) < 10 g/dL

15. Any of the following abnormal baseline laboratory tests:
   a. Serum total bilirubin > 1.5 \( \times \) ULN (upper limit of normal) (except for patients with clearly documented Gilbert’s syndrome)
   b. Alanine transaminase (ALT) or aspartate transaminase (AST) > 1.25 \( \times \) ULN
   c. Alkaline phosphatase > 2.5 \( \times \) ULN
   d. Serum creatinine > 1.5 \( \times \) ULN

16. Baseline left ventricular ejection fraction (LVEF) < 50% by echocardiography or multi-gated scintigraphic scan (MUGA).

### 5.4 Concomitant Medication and Treatment

Patients should receive full supportive care according to clinical need, the investigator’s judgment and routine clinical practice. This includes premedication and antiemetic therapy according to local guidelines, and antibiotics for the treatment of suspected infections. All concomitant medication and any diagnostic, therapeutic or surgical procedure performed during the study period should be recorded in the e-CRF (Concomitant Medication form). At the time of this protocol version, bisphosphonates are not recommended for neo-adjuvant treatment of breast cancer but may be used after definitive surgery according to their licensed indication.

Hematopoietic growth factors may be used to treat prolonged or symptomatic neutropenia but should not be used prophylactically. Similarly, patients may receive antibiotics as clinically indicated but prophylactic antibiotics should not be given routinely to all patients. Patients with anemia should be treated according to routine clinical practice and local guidelines.
guidelines. It is recommended that the hemoglobin be maintained above 10 g/dL for this trial.

The use of strong CYP3A4 inhibitors (e.g. ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, amprenavir, indinavir, nelfinavir, delavirdine, or voriconazole) is prohibited during treatment. Caution should also be used when considering administration with mild or moderate CYP3A4 inhibitors during treatment and alternative therapeutic agents that do not inhibit CYP3A4 should be considered for coadministration. Patients receiving CYP3A4 inhibitors during treatment should be monitored more closely for acute toxicities (e.g., frequent monitoring of peripheral blood counts between cycles).

If an anticoagulant therapy is required, the use of low-molecular weight heparin is recommended. Coumadin therapy is an exclusion criterion and is not allowed on trial: if prescribed during the study treatment must notify study team.

Vitamin D and calcium supplements are allowed in patients who receive aromatase inhibitors after definitive surgery.

5.5 Study withdrawal procedures
Patients have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of intercurrent illness, adverse events, disease recurrence, protocol violation, administrative or other reasons. However, an excessive rate of withdrawals could make the study uninterpretable so unnecessary withdrawal of patients should be avoided.

5.5.1 Investigator-initiated discontinuation of study therapy
In addition to the conditions outlined in the protocol, the investigator may require a patient to discontinue study therapy if one of the following occurs:

- The patient develops a serious side effect that she cannot tolerate or that cannot be controlled with other medications
- The patient’s health gets worse
- The patient is unable to meet the study requirements

If study therapy is stopped, study data should be submitted according to the study schedule unless the patient withdraws from the study (see below).

5.5.2 Patient-initiated discontinuation of study therapy
Even after a patient agrees to take part in this study, she may stop therapy or withdraw from the study at any time. If study therapy is stopped but she still allows the study doctor to submit information, study data should be submitted according to the study schedule.

5.5.3 Patient-initiated withdrawal from study
Should a patient decide to withdraw from the study, every effort should be made to complete and report the study observations as thoroughly as possible, particularly the reason for withdrawal and any underlying adverse event.

If a patient chooses to have no further interaction regarding the study, the investigator must provide written documentation of the patient’s decision to fully withdraw from the study.

A patient may decide to discontinue study treatment. This is not the same as withdrawal from the study and the patient should be asked if she would be willing to remain in the study and continue to be followed up as for other patients.
Patients who withdraw from the study after randomization will not be replaced.

5.6 Non-protocol therapy guideline
Administration of chemotherapy other than that specified in the protocol is prohibited until the time of diagnosis of first breast cancer progression during treatment or recurrence after surgery or at diagnosis of a second primary malignancy.

Administration of target therapies is prohibited until the time of diagnosis of breast cancer progression during treatment or recurrence after surgery or at diagnosis of a second primary malignancy.

5.7 Post Study Care
Patients who recur during the study should be treated according to the investigator’s clinical judgment.

6. REGISTRATION AND RANDOMIZATION

6.1 Procedures for Enrollment of Eligible Patients
Registration will be done on e-CRF and stratified randomization will be performed using a web-based randomization, accessible over a secured internet connection.

Patients who are candidates for enrollment into the study will be evaluated for eligibility by the investigator to ensure that the criteria given in Sections 3.2 and 3.3 have been satisfied, and that the patient is eligible for participation in this clinical protocol. Once a patient has fulfilled all the entry criteria, she can be registered into the protocol. The investigator or designee will then enter all patient eligibility data and information on e-CRF (Eligibility/Registration Form).

A unique patient number (UPN), without any reference to patient’s initials and birth date, will be automatically generated by EDC System for any patients registered on Eligibility/Registration Form.

Making reference to UPN, as soon as a patient has been registered the site will send, via courier and according to protocol requirements, a tumor block to the referral pathology laboratory for confirmation of HER2 status, hormone receptor status and Ki67 value.

The result of the referral laboratory will be recorded on the appropriate section of the e-CRF. A hard copy of the appropriate page, which also includes UPN, stratification variable and patient eligibility, will be sent periodically to the study site for filing in the patient’s medical record as source document, and to the Central Office of the Cooperative Research Group.

On the basis of the referral laboratory report, Michelangelo Operations Office (MOO) will:

- verify that the tests were technically feasible
- verify that negativity of local HER2 testing was confirmed
- verify that the Ki67 value is ≥ 14% in patients with ER and/or PgR positive tumors

If all the above conditions are satisfied, MOO will assign the patient to the protocol regimen. Should any of the above conditions not be satisfied, the patient will be classified as non eligible. The reason for non eligibility will be reported in the e-CRF.
6.2 Timings from Registration to Randomization

The turnaround time for the referral laboratory results should be no more than 3-5 working days from the date of sample receipt at the referral laboratory.

Within one additional working day from receipt of the referral laboratory results at MOO, the EDC System will randomize the patient according to the stratification variables. Randomization will be performed using a web-based randomization.

An automatic email to the Principal Investigator of the site (or to his/her designee) and to the Central Office of the Cooperative Research Group will then inform of the patient’s treatment allocation.

No patient must begin any study treatment prior to central randomization.

Because of the statistical considerations set forth in Section 9. (Statistical considerations and analysis plan), enrolment to either of the two tumor subtypes will be discontinued after reaching the estimated percentage and will be continued only on the other subtype to reach a total of 632 patients.

7. SCHEDULE OF ASSESSMENT AND PROCEDURES

7.1 Screening Procedures

All patients must provide written informed consent before any study specific assessments or procedures are performed. Informed consent should be asked once the report of the diagnostic core biopsy (including HER2 status, ER and PgR status and Ki67 value and/or tumor grade) is available to the site.

All screening procedures must be performed within a maximum of 4 weeks from the date of signature of Informed Consent and all eligibility criteria must be entered by the site into the e-CRF for evaluation of patient eligibility by Michelangelo.

Once all the screening data and the local pathology report data had been entered in the eCRF the tumor block, required for central laboratory review, must be shipped to the referral laboratory. If the sample shipped to the referral laboratory is insufficient and not representative of the invasive tumor component, the patient will be informed that unfortunately the tumor sample was inadequate for assessment and the patient can either
(1) Undergo a further breast biopsy, which if sufficient and meets inclusion criteria will enable the patient to be randomized onto trial; or,
(2) Not proceed with a further breast biopsy and be regarded as ineligible for the clinical trial (i.e. screen failure).

For taking truly representative core of the invasive tumor component see instructions in the Michelangelo ETNA Guidelines).

Investigations performed as part of the patient’s routine care before she has consented to the study, may be used as study assessments (with the exception of the serum or urine pregnancy test in women of child-bearing potential) of and do not need to be repeated as long as they fall within 2 weeks prior to the date of signature of Informed Consent.
Screening procedures include the following exams:

- Primary tumor core biopsy (see instructions for taking core biopsies in the Michelangelo ETNA Guidelines)
- Physical examination: height, weight, ECOG performance status, vital signs (blood pressure)
- Hematological examination: hemoglobin (Hb), white blood count (WBC) with differential, platelet count
- Biochemistry examination: serum creatinine, electrolytes (sodium, magnesium, potassium, calcium), lactate dehydrogenase (LDH), total bilirubin, alkaline phosphatase (ALP), AST/SGOT, ALT/SGPT
- Clinical (by palpation) and imaging assessment and measurement [with one of the methods in use at each clinical site: bilateral mammography or computerized tomography (CT) or magnetic resonance imaging (MRI) or ultrasound (US)] of primary breast tumor and regional nodes. It is recommended that during physical examination, while the patient lies on her back, the center of the palpable lesion in the primary tumor be marked by a tattoo (extreme of the two largest perpendicular diameters) if clips or other injectable materials were not placed at core biopsy
- HER2 (based on local testing by immunohistochemistry and/or FISH, CISH, or other amplification tests done locally)
- Hormone receptor status (ER and PgR)
- Tumor grade
- Ki67 value (if institutional standard permits)
- Imaging tests to rule out the presence of contralateral cancer or distant metastases. If institutional standards permit, chest and abdomen computerized tomography (CT) or magnetic resonance imaging (MRI) or PET. Minimal requirements are bilateral mammography, chest X-ray (postero-anterior and lateral), and abdominal ultrasound
- Bone nuclear imaging to rule out metastases is mandatory if alkaline phosphatase is > ULN or if patient has unexplained bone pain
- Medical and cardiac history, allergies, and concomitant medication(s)
- Cardiac examination: Electrocardiograph (ECG), Left ventricular ejection fraction (LVEF) by echocardiography or multi-gated scintigraphic scan (MUGA scan)
- Serum or urine pregnancy test in women of child-bearing potential (a negative serum pregnancy test must be provided within 72 hours prior starting study drug treatment)

7.2 Before and During Treatment Procedures

7.2.1 Before starting chemotherapy Mandatory for all patients

- History and assessment of Adverse Events (AE)/Serious Adverse Events(SAE) of all types and grades since date of signed informed consent
- Clinical (by palpation) assessment and measurement of primary breast tumor and regional nodes. It is recommended that during physical examination, if not done at screening or if clips were or other injectable materials not placed at core biopsy, while the patient lies on her back, the center of the palpable lesion in the primary tumor be marked by a tattoo (extreme of the two largest perpendicular diameters). Exception: according to local guidelines, identification of the primary tumor area is allowed before the second cycle of either taxane

Mandatory if more than 4 weeks have elapsed from screening examinations, recommended in other patients
• Physical examination: weight, ECOG performance status
• Hematological examination allowed within 15 days: hemoglobin (Hb), white blood count with neutrophils and platelet count
• Biochemistry examination: total bilirubin, alkaline phosphatase (ALP), AST/SGOT, ALT/SGPT.

7.2.2 Evaluations to be performed before each abraxane/paclitaxel administration (allowed within 48 hours prior to the planned treatment administration)
• History and assessment of AEs/SAEs of all types and grades, since last visit
• Hematological examination: hemoglobin (Hb), white blood count with neutrophils and platelet count

7.2.3 Evaluations to be performed before each treatment cycle (allowed within 48 hours prior to the planned treatment administration)
• Physical examination: weight, ECOG performance status
• History and assessment of AEs/SAEs of all types and grades, since last visit
• Hematological examination: hemoglobin (Hb), white blood count (WBC) with neutrophils absolute count (ANC), platelet count
• Biochemistry examination: total bilirubin, alkaline phosphatase (ALP), AST/SGOT, ALT/SGPT
• Clinical (by palpation) assessment and measurement of the primary breast tumor and regional nodes

7.2.4 Evaluations to be performed before surgery (within approximately 3 weeks from the date of last chemotherapy administration)
• Physical examination: weight, ECOG performance status
• History and assessment of AEs/SAEs of all types and grades, since last visit
• Hematological examination: hemoglobin (Hb), white blood count (WBC) with absolute neutrophils count (ANC), platelet count
• Biochemistry examination: total bilirubin, alkaline phosphatase (ALP), AST/SGOT, ALT/SGPT
• Clinical (by palpation) and imaging (using the same method at baseline) assessment and measurement of primary breast tumor and regional nodes
• Imaging tests to rule out the presence of distant metastases
• Bone nuclear imaging to rule out metastases is mandatory if alkaline phosphatase is > ULN or if patient has unexplained bone pain
• Liver imaging to rule out metastases is mandatory in patients with AST or alkaline phosphatase > ULN
• Cardiac examination: Electrocardiograph (ECG), Left ventricular ejection fraction (LVEF) by multi-gated scintigraphic scan (MUGA scan) or echocardiography

7.3 End of Study Treatment Procedures (4-5 weeks, i.e. 28-35 days, from the date of surgery)
For the purposes of the protocol, the end of protocol treatment is defined as the date of surgery. The following assessments must be performed:
• Physical examination: weight, ECOG performance status
• History and assessment of AEs/SAEs of all types and grades, since last visit
• Hematological examination: hemoglobin (Hb), white blood count (WBC) with differential, platelet count
• Biochemistry examination: serum creatinine, electrolytes (sodium, magnesium, potassium, calcium), lactate dehydrogenase (LDH), total bilirubin, alkaline phosphatase (ALP), AST/SGOT, ALT/SGPT

7.4 Follow-up procedures
Although the primary aim of the study is the rate of pathological complete remission, in order to collect sound data on the secondary end-points of DFS, DDFS, LRFS, RRFS and OS, all patients will be followed for 10 years after the date of randomization for the last participant. Recommendations on timing of examinations will be according to conventional local rules.

The following investigations are suggested yearly, investigators may wish to see their patients more frequently according to their routine practice:
• Physical examination and assessment of possible tumor recurrence
• Mammography (bilateral in case of conservative surgery)
• Treatment related AE assessment
• Hematological and biochemistry examinations
• Imaging tests to rule out the presence of distant metastases
• Bone nuclear imaging to rule out metastases is mandatory if alkaline phosphatase is > ULN or if patient has unexplained bone pain
• Liver imaging to rule out metastases is mandatory in patients with AST or alkaline phosphatase > ULN
• Cardiac examination: Electrocardiograph (ECG), Left ventricular ejection fraction (LVEF) by echography or multi-gated scintigraphic scan (MUGA scan) for the first 2 years

A brief summary of the investigation reports must be entered yearly in the e-CRF to assess any change in the patient’s status during follow-up

7.5 Diagnosis of breast cancer progression during treatment or recurrence after definite surgery
The diagnosis of a breast cancer progression or recurrence can be made only when the clinical, laboratory, radiological and/or histological findings meet the criteria of ‘acceptable’ as defined below. Suspicious findings do not constitute criteria for breast cancer recurrence, nor are they an indication to alter protocol therapy.

Treatment of a breast cancer recurrence or second primary cancer will be at the discretion of the investigator.

7.5.1 Local recurrence/local progression
Local recurrence is defined as evidence of breast cancer (invasive of in situ) in the ipsilateral breast or skin of the breast after surgery. When local recurrence occurs in the ipsilateral breast parenchyma after conservative surgery it is termed ‘Ipsilateral breast tumor recurrence (IBTR)’. Patients who develop clinical evidence of tumor recurrence in the remainder of the ipsilateral breast must have a biopsy of the suspicious lesion to confirm the diagnosis.
• Acceptable: Positive cytology or histological biopsy

Local progression is defined as increase of tumor size under treatment (during primary chemotherapy)
• Acceptable: Positive cytology or histological biopsy, imaging

Other local recurrence/progression
Defined as appearance of breast cancer in the skin of the chest wall (exclusive of the breast) or chest wall after surgery (recurrence) or during primary chemotherapy (progression)
7.5.2 **Regional recurrence/Regional progression**
Defined as increase in size or new appearance of tumor in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes, as well as the soft tissue of the ipsilateral axilla, following surgery. 
Acceptable: Positive cytology or histological biopsy.

7.5.3 **Contralateral invasive breast cancer**
Defined as evidence of invasive breast cancer in the contra lateral breast or chest wall. The diagnosis of contralateral breast cancer must be confirmed histologically. 
- Acceptable: Positive biopsy or cytology

7.5.4 **Distant recurrence/Distant progression**
Defined as evidence of tumor in all areas, with the exception of those described in the previous sections.

*Skin, subcutaneous tissue, and lymph nodes (other than local or regional)*
Acceptable: Positive cytology, aspirate or biopsy, or radiologic evidence of metastatic disease.

*Bone marrow metastasis*
Acceptable: Positive cytology, biopsy, or MRI scan.

*Lung metastasis*
- Acceptable: Positive cytology, or biopsy or radiologic evidence of multiple pulmonary nodules that are judged to be consistent with pulmonary metastases. 
  *Note:* If a solitary lung lesion is found and no other lesions are present on lung tomograms, CT scan, or MRI scan, further investigations, such as biopsy, needle aspiration, or PET scan must be performed. Proof of neoplastic pleural effusion must be established by cytology or pleural biopsy.

*Skeletal metastasis*
- Acceptable: X-ray, CT, or MRI evidence of lytic or blastic lesions consistent with bone metastasis, or bone scan that is clearly positive for bone metastases. 
  *Note:* If the diagnosis is equivocal by bone scan or radiologic evaluation, a biopsy is strongly recommended. Any positive bone scan in joints or in a recent area of trauma (surgical or otherwise) cannot be used as a criterion for breast cancer recurrence.

*Liver metastasis*
- Acceptable: an abdominal CT scan, liver scan, ultrasound, or MRI consistent with liver metastases or liver biopsy confirmation of the metastatic disease. 
  *Note:* If the radiologic findings are not definitive (especially with solitary liver nodules), a liver biopsy is recommended; however, if a biopsy is not performed, serial scans must be obtained to document stability or progression.

*Central nervous system*
• Acceptable: Positive CT scan or MRI scan, usually in a patient with neurological symptoms, or biopsy or cytology (for a diagnosis of leptomeningeal involvement).

8. TREATMENT

8.1 Neoadjuvant chemotherapy

Neoadjuvant chemotherapy should start as soon as possible after treatment assignment and not more than 5 working days later.

Doses for all cytotoxic drugs will be based on the patient’s baseline body surface area (BSA). In calculating BSA, actual weights and heights should be used. No downward adjustments for “ideal body weight” are recommended. This applies to all patients whose calculated BSA is ≤ 2.2 m². In patients with a calculated BSA > 2.2 m², a BSA of 2.2 m² should be used. The BSA does not need to be recalculated during the treatment phase unless there is a significant (>10%) loss or increase of body weight compared to baseline.

The planned cytotoxic treatments are potentially emetogenic. Local investigators are responsible for deciding the most appropriate measures to prevent nausea and vomiting.

8.1.1 Abraxane

- Premedication

Hypersensitivity reactions rarely occur with abraxane and premedication is not commonly required. However, should the patient has a history of any allergies, the course of action to be taken is at investigator’s discretion.

Prophylactic antiemetic medication with 5-HT3 (p.o. or i.v.) is recommended prior to and/or following abraxane administration. Antiemetic and anti-allergic reaction prophylaxis with steroids prior to abraxane administration is not recommended.

- Treatment

Abraxane will be delivered as intravenous infusion at the dose of 125 mg/m² over 30 minutes given week 1, 2 and 3 followed by 1 week rest.

If hypersensitivity occurs, abraxane should be discontinued immediately and symptomatic treatment should be initiated.

- Number of cycles and cycle duration

Cycles of abraxane will be repeated every 28 days (± 2 days), toxicity permitting, for a total of 4 consecutive cycles.

- Dose modifications for abraxane

Treatment should be delayed for at least 1 week for an absolute neutrophil count less than 1.0 x 10⁹/L and/or a platelet count less than 100 x 10⁹/L and treatment-related non-hematological toxicity has resolved to ≤ Grade 1 (except for G 2 alopecia and fatigue for which resolution is not required). Once the dose at the start of a treatment cycle has been reduced, no re-escalation is permitted. Should neutrophil count and/or platelet count persist below the above mentioned value for > 14 days, please contact the headquarters of the Cooperative Research Group or MOO.
Recommendations for abraxane dose interruptions/modifications in case of specific treatment-emergent AEs are provided in the following sections.

As a general rule, if dose reduction of abraxane is necessary, the dose should be reduced by one dose level, and the subject should be monitored for 10 to 14 days at each dose level. If toxicity does not abate during this monitoring time, abraxane may need to be interrupted with continued monitoring for an additional 10-14 days.

Once the dose has been reduced no re escalation is allowed.

If a subject’s treatment has been interrupted for more than 21 days, the investigator must contact the headquarters of the Cooperative Research Group or MOO to review the subject’s condition in order to resume the treatment.

<table>
<thead>
<tr>
<th></th>
<th>Abraxane (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (starting dose)</td>
<td>125</td>
</tr>
<tr>
<td>-1</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5: Abraxane Dose Levels

- Dose reductions and guidelines for frequent adverse events of abraxane

Patients experiencing any of the following toxicities during the previous cycle should have their chemotherapy reduced for all subsequent cycles by 1 dose level (by approximately 20% of the previous dose) as outlined on Table 6.
<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Abraxane</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC ≤ 0.5 x 10^9/L for ≥ 5 days</td>
<td>Withheld till recovery(^{(b)}) and decrease 1 level</td>
</tr>
<tr>
<td>Febrile neutropenia (≥ 38.5°C) associated with ANC &lt; 1.0 x 10^9/L</td>
<td>Withheld till recovery(^{(b)}) and decrease 1 level</td>
</tr>
<tr>
<td>≥ Grade 3 thrombocytopenia or in the presence of significant bleeding or requiring blood transfusion \textit{at first occurrence}</td>
<td>Withheld till recovery(^{(b)}) and decrease 1 level</td>
</tr>
<tr>
<td>Grade 2 sensory neuropathy lasting &gt; 7 days</td>
<td>Withheld till neuropathy improves to ≤ Grade 1 and decrease 1 level</td>
</tr>
<tr>
<td>Grade 3 sensory neuropathy</td>
<td>Withheld Treatment may be resumed at a reduction of 1 level in subsequent cycles after neuropathy improves to ≤ Grade 1</td>
</tr>
<tr>
<td>Grade 4 sensory neuropathy</td>
<td>Withheld Treatment may be resumed at a reduction of 1 level in subsequent cycles after neuropathy improves to ≤ Grade 1 If neuropathy does not improve to ≤ Grade 1 within 6 weeks discontinue treatment</td>
</tr>
<tr>
<td>Abnormal bilirubin value</td>
<td>Re-test bilirubin every week, continue study treatment</td>
</tr>
<tr>
<td>- Grade 1</td>
<td>Hold abraxane until improvement to Grade 1. Re-start abraxane at a lower dose level Discontinue abraxane</td>
</tr>
<tr>
<td>- Grade 2</td>
<td></td>
</tr>
<tr>
<td>- Grade 3 or 4</td>
<td></td>
</tr>
<tr>
<td>Abnormal AST/ALT values</td>
<td>Continue study treatment</td>
</tr>
<tr>
<td>- Grade 1</td>
<td>Hold abraxane until improvement to Grade 1. Re-start abraxane at a lower dose level Discontinue abraxane</td>
</tr>
<tr>
<td>- Grade 2</td>
<td></td>
</tr>
<tr>
<td>- Grade 3 or 4</td>
<td></td>
</tr>
<tr>
<td>Other Grade ≥ 3 toxicities(^{c})</td>
<td>Adjust dose or discontinue therapy as medically indicated after discussion with the headquarters of the Cooperative Research Group or MOO</td>
</tr>
</tbody>
</table>

\(^{a}\) Despite adequate/maximal medical intervention and/or prophylaxis.

\(^{b}\) Neutrophils have to recover to ≥ 1.5 and platelets have to recover to ≥ 100 x 10^9/L before the start of the next cycle. If platelets and/or neutrophils have not recovered on day 35, discontinue treatment

\(^{c}\) Except Grade 3 fatigue, transient joint or muscle pain for which no dose modifications are required.
- **Hypersensitivity Reactions**

Hypersensitivity reactions rarely occur. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea, lower back pain, hypotension, or tachycardia may require temporary interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticaria require immediate discontinuation of study drug administration and aggressive symptomatic therapy. Patients who experience a severe hypersensitivity reactions to abraxane should not be re-challenged.

### 8.1.2 Paclitaxel

- **Premedication**

All patients must receive pre-medication according to local guidelines. The following scheme is only an indication:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>250 mg</td>
<td>i.v</td>
<td>30 minutes prior to paclitaxel</td>
</tr>
<tr>
<td>Chlorphenamine</td>
<td>10 mg</td>
<td>i.v or i.m.</td>
<td>30 minutes prior to paclitaxel</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>300 mg</td>
<td>i.v</td>
<td>30 minutes prior to paclitaxel</td>
</tr>
</tbody>
</table>

*Table 7: Premedication*

Alternative drugs may be used if those listed in the table are not commercially available. The alternative premedication must be kept constant throughout the trial.

- **Treatment**

At the end of pre-medication patients will receive paclitaxel at the dose of 90 mg/m² diluted in 250 mL as per Summary of Product Characteristics (SmPC) over 1 hour given week 1,2 and 3 followed by 1 week rest. Some patients may experience asymptomatic bradycardia during paclitaxel infusion. Furthermore, hypersensitivity reactions are possible and usually occur within the first hour from the beginning of the infusion in the first and (less frequently) the second cycle. For these reasons, *it is recommended that patients’ blood pressure is monitored during the infusion and that patients are supervised for at least one hour after the end of paclitaxel administration.*

- **Number of cycles and cycle duration**

Cycles of paclitaxel will be repeated every 28 days (± 2 days), toxicity permitting, for a total of 4 consecutive cycles.

- **Dose modification for paclitaxel**

Treatment should be delayed for at least 1 week for an absolute neutrophil count less than 1.0 x 10^9/L and/or a platelet count less than 100 x 10^9/L and treatment-related non-hematological toxicity has resolved to ≤ Grade 1 (except for G 2 alopecia and fatigue for which resolution is not required). Once the dose at the start of a treatment cycle has been reduced, no re-escalation is permitted. Should neutrophil count and/or platelet count persist below the above mentioned value for > 14 days, please contact the headquarters of the Cooperative Research Group or MOO.
Recommendations for paclitaxel dose interruptions/modifications in case of specific treatment-emergent AEs are provided in the following sections.

As a general rule, if dose reduction of paclitaxel is necessary, the dose should be reduced by one dose level, and the subject should be monitored for 10 to 14 days at each dose level. If toxicity does not abate during this monitoring time, paclitaxel may need to be interrupted with continued monitoring for an additional 10-14 days at each dose level, and so on.

Once the dose has been reduced no reescalation is allowed.

If a subject’s treatment has been interrupted for more than 21 days, the investigator must contact the headquarters of the Cooperative Research Group or MOO to review the subject’s condition in order to resume the treatment.

**Paclitaxel 90 mg/m² Dose Levels**

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Paclitaxel (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (starting dose)</td>
<td>90</td>
</tr>
<tr>
<td>-1</td>
<td>75</td>
</tr>
</tbody>
</table>

*Table 8: Paclitaxel Dose Levels*

---

**Dose reductions and guidelines for frequent adverse events of paclitaxel**

Patients experiencing any of the following toxicities during the previous cycle should have their chemotherapy reduced for all subsequent cycles by 1 dose level (by approximately 20% of the previous dose) as outlined on Table 9.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC ≤ &lt;0.5 x 10⁹/L for ≥ 5 days</td>
<td>Withheld till recovery&lt;sup&gt;(b)&lt;/sup&gt; and decrease 1 level</td>
</tr>
<tr>
<td>Febrile neutropenia (≥ 38.5°C) associated with ANC &lt; 1.0 x 10⁹/L</td>
<td>Withheld till recovery&lt;sup&gt;(b)&lt;/sup&gt; and decrease 1 level</td>
</tr>
<tr>
<td>≥ Grade 3 thrombocytopenia or in the presence of significant bleeding or requiring blood transfusion</td>
<td>Withheld till recovery&lt;sup&gt;(b)&lt;/sup&gt; and decrease 1 level</td>
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<tr>
<td>Grade 4 sensory neuropathy</td>
<td>Withheld&lt;br&gt;Treatment may be resumed at a reduction of 1 level in subsequent cycles after neuropathy improves to ≤ Grade 1&lt;br&gt;If neuropathy does not improve to ≤ Grade 1 within 6 weeks discontinue treatment</td>
</tr>
</tbody>
</table>
Table 9: Dose Reductions for paclitaxel

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Grade ≥ 3 toxicities[^c]</td>
<td>Adjust dose or discontinue therapy as medically indicated after discussion with the headquarters of the Cooperative Research Group or MOO</td>
</tr>
</tbody>
</table>

[^a] Despite adequate/maximal medical intervention and/or prophylaxis.

[^b] Neutrophils have to recover to ≥ 1.5 and platelets have to recover to ≥ 100 x 10[^9]/L before the start of the next cycle. If platelets and/or neutrophils have not recovered on day 35, discontinue treatment.

[^c] Except Grade 3 fatigue, transient joint or muscle pain for which no dose modifications are required.

**Hypersensitivity Reactions**

Paclitaxel can cause hypersensitivity reaction in spite of pre-medication. In case of any grade of hypersensitivity (except for skin redness with butterfly distribution involving the face when close monitoring is advised with interrupting therapy) paclitaxel infusion should be immediately discontinued.

The following management of hypersensitivity reactions is recommended:

i. Administer chlorphenamine 10 mg i.v.

ii. If hypotension is present, administer i.v. fluids.

iii. If hypotension persists and/or wheezing is present, administer adrenaline (or its equivalent) s.c. every 15-20 min. until reaction subsides.

iv. If wheezing is present that does not respond to adrenaline (or its equivalent), administration of 0.35 cc of nebulized albuterol solution (or its equivalent) is recommended.

v. Although corticosteroids have no effect on the initial reaction, they have been shown to block “late” allergic reactions to a variety of substances. Thus, methylprednisolone 125 mg i.v. (or its equivalent) may be administered to prevent recurrent or ongoing allergic reactions.

Patients with CTCAE grade 3 or 4 infusion reactions should permanently discontinue paclitaxel.

At the investigator’s discretion, patients with CTCAE grade 2 hypersensitivity reactions requiring interruption of a paclitaxel infusion may be re-challenged with paclitaxel according to the following protocol:

i. Dexamethasone (no substitution for dexamethasone should be performed) 8 mg per dose i.v. 24, 18, 12, 6 hours prior to paclitaxel;

ii. Chlorphenamine, 10 mg i.v. 30 minutes prior to paclitaxel;

iii. Cimetidine 300 mg i.v. 30 minutes prior to paclitaxel;

iv. Give paclitaxel in 1000 mL. Patients should be carefully monitored (i.e. vital signs every 15 min. during the 1st hour and every 30 min. for the 2nd and 3rd hour, then once an hour). Initial infusion at low rate for first 30 min., then back to normal.

### 8.1.3 AC or EC (doxorubicin or epirubicin and cyclophosphamide)

AC will be started between day 14 and day 21 after the last administration of abraxane or paclitaxel (toxicity permitting) according to the following doses and schedule.
- **Treatment**

Doxorubicin will be administered at the dose of 60 mg/m\(^2\) diluted in 40 mL of normal saline and infused in no more than 15 min. through the side-port of a rapidly running intravenous infusion. It is recommended that the vein is infused with 20-30 mL of NS or D5W immediately afterwards. Other dilution and infusion procedures as per local standard are allowed.

Alternatively epirubicin at the dose of 90 mg/m\(^2\) is allowed in substitution for doxorubicin.

Cyclophosphamide will be administered at the dose of 600 mg/m\(^2\) diluted in 40 mL of normal saline and infused in no more than 15 min. through the side-port of a rapidly running intravenous infusion. Other dilution and infusion procedures as per local standard are allowed.

- **Number of cycles and cycle duration**

Cycles of AC or EC will be repeated every 21 days (± 2 days), toxicity permitting, for a total of 4 consecutive cycles. Dose delays and dose reductions for toxicity are permitted. Please refer to local prescribing information about possible adverse events and dose modifications.

The maximum cumulative dose of doxorubicin is 240 mg/m\(^2\). At this cumulative dose cardiac effects are rare (< 2%). Doxorubicin will be discontinued if

1. congestive heart failure appears;
2. persistent arrhythmia (including sinus tachycardia with no demonstrable cause) appears;
3. asymptomatic decrease of LVEF to below 45%

### 8.1.4 FEC (fluorouracil, epirubicin, cyclophosphamide)

FEC will be started between day 14 and day 21 after the last administration of abraxane or paclitaxel (toxicity permitting) according to the following doses and schedule.

- **Treatment**

Patients will receive 5-fluorouracil (600 mg/m\(^2\) by bolus injection), epirubicin (90 mg/m\(^2\) diluted in up to 100mL WFI or saline and infused over 15 minutes through the side port of a rapidly running intravenous infusion) and cyclophosphamide (600 mg/m\(^2\) by bolus injection). Other dilution and infusion procedures as per local standard are allowed

- **Number of cycles and cycle duration**

Cycles of FEC will be repeated every 21 days (± 2 days), toxicity permitting, for a total of 4 consecutive cycles. Dose delays and dose reductions for toxicity are permitted. Please refer to local prescribing information about possible adverse events and dose modifications.

### 8.2 Locoregional treatment

At the completion of neoadjuvant chemotherapy all patients must receive local-regional treatment (breast conserving surgery and radiotherapy, or mastectomy with or without postmastectomy radiotherapy, consistent with current standards of care).

#### 8.2.1 Surgery

a. Surgery should be performed within 3 to 5 weeks from the last dose of preoperative therapy, according to procedures decided by the local surgeon who should take into account tumor size, breast volume, and patient's attitude.
b. **Breast surgery**
Whenever possible, in operable and locally advanced non-inflammatory cancers conservation methods should be preferred. Conservation should consist of a wide resection, with safe free margins. **Definition of safe free margins is the one adopted in the Participating Center or in the NCCN guidelines, version 2012.**

**Conservative surgery is not recommended for inflammatory breast cancer** or in the presence of microcalcifications. After mastectomy, breast reconstruction with implants may be considered only after assessment and information of risks related to the subsequent radiotherapy on the chest wall. Its feasibility should be agreed upon with the radiation therapist.

c. **Axillary surgery**
Axillary lymph node dissection (ALND) is recommended because the definition of the primary aim is pCR in breast and axillary nodes. Lymph node axillary dissection must include the first and second level. Sentinel node biopsy is allowed in cN0 before neoadjuvant therapy or in cN0 after neoadjuvant therapy and 3 to 5 “sentinel nodes” are recommended to minimize the false negative rate. Sentinel node biopsy must be followed by ALND if positive nodes are found.

- **Pathology assessments**
The primary endpoint of this trial is the rate of pathologic Complete Response (pCR), determined as ypT0/ypTis, ypN0 according to AJCC classification. Therefore, accurate and reproducible assessment of pCR is very important. Although not mandatory, the collection of the following data to evaluate the Residual Cancer Burden (RCB) is highly recommended:

- primary tumor bed area (... mm x ... mm)
  - “tumor bed” characteristic findings are: fibrotic “scar” tissue, lymphocytic infiltration, groups of foamy cells, lack of glandular tissue
  - IHC for cytokeratins may be used if necessary to assist in the identification of epithelial (malignant) cells
- overall cellularity of residual primary tumor (as a percentage of area)
- percentage of in situ component
- number of positive nodes
- size of the largest metastasis

All instructions for the definition of pCR and RCB are given in the Michelangelo Guidelines.

### 8.2.2 Radiotherapy
All patients subjected to breast-conserving surgery will receive postoperative irradiation. Treatment should start within 4 to 6 weeks after surgery. The breast should be irradiated with two opposing tangential fields and treatment should consist of 5 fractions a week to a total dose of 50 Gy. A boost of 10 Gy in 5 fractions may be given to the tumor bed. **No partial breast irradiation is allowed.**

After mastectomy, the irradiation of the chest wall with electrons is mandatory for pT4 cases. Total suggested dose is around 45 Gy.

For patients with locally advanced or inflammatory breast cancer irradiation should be delivered according to international and local guidelines.

### 8.2.3 Endocrine therapy after definite surgery
Patients with ER and/or PgR positive disease must receive endocrine therapy on completion of neoadjuvant chemotherapy and surgery.
The investigator’s choice of hormone therapy for each individual patient must be decided according to local guideline and documented in the e-CRF. Patients who receive aromatase inhibitors may also receive vitamin D, calcium supplements and biphosphonates.

9. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

9.1 Sample size considerations
The primary endpoint is to compare the rate of pathologic Complete Response (pCR) between abraxane and paclitaxel. Treatment contrast is expressed in terms of relative measure of effect, i.e. an odds ratio (OR) equal to 1.7, corresponding to an absolute difference of 10% when the pCR for paclitaxel is 20%, setting a target pCR for abraxane to 30%.

The derived OR is then used to employ a two sided Cochran-Maentel Hanszel design, stratifying the comparison between the 2 arms by breast cancer phenotype (triple negative, others).

Assuming to screen 40% patients triple negative and 60% patients with other tumors, with an estimated response rate to paclitaxel of 32% and 15%, respectively, 632 patients (316 per arm) are required to reject the OR set by the null hypothesis of 1 when the OR is actually 1.7, with power=80% and the false positive rate=5%.

The expected overall pathological complete response rate in breast and axilla (pCR) for HER2- patients with operable and locally advanced breast cancer, that is 20%, is taken from results of the GeparTrio study where patients were essentially treated with a docetaxel containing regimen. In the GeparTrio trial, in a sample of about 1000 HER2- patients, a pCR at surgery was recorded for 20% of them. The recent updated analysis presented at the San Antonio Breast Cancer Conference reported a pCR for 133 patients out of 362 triple negative patients (36.7%) and for 39 patients out of 211 luminal B patients (18.5%). Estimates of paclitaxel effect in the 2 study sub-populations (triple negative vs other) are based on the NOAH study in locally advanced breast cancers. In the NOAH, in the limited parallel group of HER2-negative tumors 11 patients out of 35 triple negative patients (31%) and 2 patients out of 16 in the other tumors (12.5%) attained a pCR.

Because of the above mentioned statistical considerations, enrolment to either of the two tumor subtypes will be discontinued after reaching the estimated percentage and will be continued only on the other subtype to reach a total of 632 patients.

Sample size computations were made using Pass 2008 software.

9.2 Analysis populations

Intent-to-Treat Population (ITT)
The primary population for all efficacy endpoints will be the ITT (intent-to-treat) population, defined as all randomized patients classified on the basis of the treatment arm they were randomized to.

Per Protocol Population (PP)
The PP population is defined as the ITT population who completed the scheduled courses of treatment, without any major protocol violation regarding the efficacy evaluation and any other reason for exclusion from efficacy analysis.

Detection of any of the following items will exclude the patient from the PP population:
- Evidence of bilateral invasive breast cancer or metastatic disease (M1)
- Any previous therapy for breast cancer, including chemotherapy, hormonal therapy and radiotherapy
• Hematological and/or biochemical values not meeting the eligibility criteria, as specified in the exclusion criteria section
• Missing evaluation of pathological complete response at surgery

The primary endpoint and the key secondary endpoint, i.e. event free survival and distant event free survival, will be also analyzed in the PP population, integrating the results observed in the primary population.

Safety Population
The safety population is defined as all randomized patients who received at least one dose of either abraxane or paclitaxel. Patients will be classified according to the treatment administered, regardless of any discrepancy with respect to the treatment they were randomized to.
The safety population will be used for all safety analyses.

9.3 Patient disposition, demography and baseline characteristics
Frequency distributions will be provided for patients who completed the study treatment, patients who discontinued treatment as well as for reasons for discontinuations.
Number and percentages of patients across each study population will be provided.

The following demographic variables will be summarized: age (years), age category (to be discussed with IDMC) and ethnic group. Age will be calculated at the date of randomization, using date of birth information.

Disease stage
Appropriate statistics will be calculated for: time from diagnosis of primary breast cancer to randomization, TNM staging, T stage alone, lymph node status (N0, N1, N2, N3), median tumor size, disease stage (operable, locally advanced).

Data collected from local and centralized core biopsy
Tumor grade (low, intermediate, high), histology (ductal invasive, lobular invasive, other), hormone receptor status (ER positive and/or PR positive, ER and PR both negative), Ki67 (analyzed as both continuous variables and according to the following categories: <= 14%, > 14%), tumor subtype (luminal B intermediate, luminal B high, triple negative) will be analyzed by means of frequency tables.

Medical history will be coded by MedDRA dictionary and described by System Organ Class and Preferred Term; Cardiac history will be analyzed according to the CRF preprinted categories.

As far as physical examination is concerned summary statistics will be provided for weight, height, BSA, ECOG performance status, systolic and diastolic blood pressure.

The ITT population will be used for this analysis.

9.4 Efficacy

9.4.1 Primary endpoint: Pathological Complete Response (pCR)
The primary endpoint is to compare the rate of pCR at surgery between abraxane and paclitaxel containing regimens.
Pathological complete response is defined as absence of invasive disease in breast and nodes (ypT0/ypTis, ypN0).
Absolute and relative frequencies along with exact 95% confidence intervals (Clopper-Pearson method) for the pCR rate will be provided by treatment arm. The comparison among treatments will be carried out using a two-sided Cochran-Mantel-Haenszel test, controlling for tumor sub-group (triple negative and the 2 luminal B categories pooled together) and disease stage (operable vs. locally advanced); assuming no interaction between treatments and the stratification factors, the pooled effect will be estimated by a unique odds ratio (OR) and its 95% confidence interval; the corresponding p-value will be also reported. Should the Breslow-Day test for homogeneity of the OR across strata turn out to be significant, ORs will be also presented within each combination of the levels of the stratification variables along with 95% confidence intervals, with the meaning that an interaction is present.

Treatment effect will be also expressed as absolute difference in pCR rates between treatments. 95% confidence intervals will be calculated according to the Wald method.

Exploratory sub-group analysis will be conducted within each level of stratification factors (i.e. cooperative research group, tumor sub-type, disease stage) and within each combination therapy (AC/EC and FEC): treatment effect will be tested by a two-sided chi-square test, absolute difference in pCR rates, ORs and 95% confidence intervals will be reported. The net effect of treatment adjusted for stratification factors taken all at once (i.e. all included in the model) will be evaluated by a multivariate logistic regression model. Additional prognostic factors deemed worthy of investigation to better characterize the efficacy profile of the study treatment will be identified in the SAP.

Assuming an escalating accrual rate in the first 6 months of the trial and a uniform accrual thereafter, the total enrolment of 632 patients will results in completion of enrolment in approximately 30 months. The primary analysis will be performed approximately 40 months after the randomization of the first patient, that is when all the 632 patients required by sample size specifications have completed their courses of treatment and reached the surgery phase.

### 9.4.2 Secondary efficacy endpoints

- **pCR rate in the two main subgroups of ER and/or PgR positive tumors and triple-negative tumors**

  pCR rate will be analyzed separately by a two-sided chi square test, as part of the exploratory analyses for the primary endpoint as stated above. Due to the exploratory nature of this endpoint, no alpha error adjustment will be made.

- **Clinical response: Objective Response Rate (ORR)**

  For the purpose of this study, all clinical measurements will be assessed by palpation of the breast and the axilla. The objective response rate is defined as the proportion of patients who attain either a complete response or a partial response during the study, evaluated according to RECIST criteria (v 1.1) purposely modified for this protocol and detailed in the SAP.

  Treatment comparison will be carried out by Cochran-Mantel-Haenszel, using tumor sub-group and disease stage as stratification factors; exact 95% confidence intervals for ORR will be reported within each arm.

  The strength of the association between treatment and outcome of interest will be measured by both OR and absolute difference in response rates, with their 95% confidence intervals.
- **Event Free Survival**

For the purpose of this protocol, event free survival (EFS) is defined as the time from randomization to the first date of disease progression while on primary therapy or disease recurrence (local, regional, distant, invasive contralateral breast) after surgery or death due to any cause. Patients who terminate the study without evidence of any of the above events will be censored at the date of their last follow-up tumor assessment. Patients who start a new anti-tumor therapy (with the exception of adjuvant endocrine therapy in ER or PgR positive tumors after surgery) in the absence of disease progression or recurrence will be censored at their last follow-up tumor assessment before the start of the new therapy. EFS will be analyzed by the Kaplan-Meier (KM) method; quartiles estimates of EFS along with 95% CI and KM curves will be provided. Differences in the EFS distribution between abraxane and paclitaxel will be tested by a log-rank test, stratified by tumor sub-group and disease stage.

A Cox proportional hazard model will be used to obtain hazard ratios and 95% confidence intervals for the treatment effect on EFS controlling for molecular sub-group as potential effect modifier.

Due to the exploratory nature of this endpoint no alpha adjustment will be made on repeated testing over time.

- **Distant Event Free Survival**

The distant event free survival (DEFS) is defined as the time from randomization to the first date of distant metastasis while on primary therapy or distant recurrence after surgery or death due to any cause. Patients who terminate the study without evidence of any of the above events will be censored at the date of their last follow-up tumor assessment. Patients who start a new anti-tumor therapy (with the exception of adjuvant endocrine therapy in ER or PgR positive tumors after surgery) in the absence of distant disease will be censored at their last follow-up tumor assessment before the start of the new therapy. DEFS will be analyzed by the Kaplan-Meier (KM) method; quartiles estimates of DEFS along with 95% CI and KM curves will be provided. Differences in the DEFS distribution between abraxane and paclitaxel will be tested by a log-rank test, stratified by tumor sub-group and disease stage.

A Cox proportional hazard model will be used to obtain hazard ratios and 95% confidence intervals for the treatment effect on DEFS controlling for molecular sub-group and disease stage as potential effect modifiers. With regard to the timing of analysis, the same rule as specified above for EFS will be applied.

- **Local Event Free Survival**

The local event free survival (LEFS) is defined as the time from randomization to the first date of local progression while on primary therapy or local recurrence after surgery. Rules for censoring and methods of analysis will be the same as defined for EFS.

- **Regional Event Free Survival**

The regional event free survival (REFS) is defined as the time from randomization to the first date of regional progression while on primary therapy or regional recurrence after surgery. Rules for censoring and methods of analysis will be the same as defined for EFS.

- **Overall Survival**

The overall survival (OS) is defined as the time from randomization to the date of death. Patients alive at the end of study will be censored at their last contact date. OS will analyzed by the Kaplan-Meier method; quartiles estimates of OS along with 95% CI and KM curves will be provided. Differences in the survival distribution between abraxane and paclitaxel will be tested by a log-rank test, stratified by tumor sub-group and disease stage.
A Cox proportional hazard model will be used to obtain hazard ratios and 95% confidence intervals for the treatment effect on OS controlling for molecular sub-group and disease stage as potential effect modifiers.

With regard to the timing of analysis, the same rule as specified above for EFS and DEFS will be applied.

9.4.3 Handling of missing values

Patients who drop out of the study before surgery will be included in the primary analysis, following the ITT principle, and considered as non-responder as well as randomized patients who are never treated, whatever is the reason for not receiving the treatment.

Patients with missing evaluation of pathological complete response at surgery will be included in the primary analysis and analyzed as non-responder.

With regard to the objective response rate, the same rules as defined for the primary endpoint will be applied.

As far as time to event endpoints are concerned (i.e. EFS, DEFS, LEFS, REFS OS), the ITT analysis will include all randomized patients, without any distinction.

9.5 Extent of exposure

The analysis of extent of exposure will focus on treatment duration, number of cycles administered, total cumulative dose, absolute dose intensity, relative dose intensity (compliance); drug modifications, i.e. dose reductions and delays, as well as reasons for modification will be documented.

The analysis will be presented broken down by arm and treatment received (abraxane or paclitaxel, FEC or AC), on the safety population.

9.6 Clinical Study Report (CSR)

The CSR will be written when the evaluation of the primary endpoint pCR takes place, approximately 40 months after the randomization of the first patient. The first analysis of EFS will take place 5 years after the randomization of the first patient and data will be reported in the CRS as a first addendum. At this early stage it’s very likely to have many censored values for this endpoint, i.e. most of the patients will not have experienced the event of interest. In order to get more mature data, each randomized patient will be followed until the occurrence of the event up to 10 years after randomization. When the time frame is completed the final analysis of EFS will be performed, jointly with the analysis of overall survival, and reported as a final addendum.

9.7 Safety

Patients will be assessed for adverse events by clinical examination, questioning for symptoms of toxicity, laboratory assessments, vital signs, ECG and LVEF.

Neurological toxicity and other toxicities will be assessed throughout the study according the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Event (CTCAE) Version 4.0.

Adverse events (AEs) will be coded by MedDRA dictionary and the Preferred Term will be used for reporting purposes. The analysis will address the Treatment Emergent Adverse Events (TEAE) defined as all events with onset date posterior to the date of first treatment administration or started before the first study drug administration but worsening in severity during the treatment period. For each treatment arm, the incidence of AEs will be grouped by System Organ Class (SOC) and by Preferred Term. Each patient will be counted once according to the worst grade reported throughout the whole treatment period for each SOC.
and/or for each preferred term. If clinically indicated, selected AEs will be presented by treatment cycle. Subset of relevant events such as serious AEs, AEs with severity grade 3-5, AEs leading to discontinuation of study drug, and AEs with a relationship to study treatment, will be analyzed and reported separately.

Frequency of deaths by treatment arm will be presented. Deaths will also be described in terms of relationship to the study treatment and according to the time since treatment discontinuation.

Laboratory data will be graded according to the NCI CTCAE version 4.0 scale whenever possible. For each laboratory parameter included in the NCI CTCAE system, the incidence of abnormalities will be evaluated by considering the worst CTCAE grade attained for each patient throughout the whole treatment period. Changes vs baseline will be evaluated by shift tables. For the laboratory data not graded by the NCI CTCAE scale, a cross tabulation of on treatment worst finding vs. baseline finding will be presented reporting the number of patients with values within or out of the normal range.

The incidence of ECG abnormalities after treatment initiation will be provided, possibly grouping by type of abnormality.

LVEF data will be described in terms of the worst absolute decrease from baseline (if any) after treatment start; summary statistics for continuous variables will be reported. Patients with LVEF values below 50% and 45% will be identified.

All collected safety data (including vital signs, ECG, LVEF, laboratory assessments and adverse events) will be also presented in individual patients’ data listings.

An Independent Data Monitoring Committee will monitor patient safety at pre-specified times as well as at ad hoc meeting if requested.

**9.8 Responsibility for analysis**

All the efficacy and safety analyses of the study will be under the responsibility of Fondazione Michelangelo and they will be based on a statistical plan according to the protocol pre-specified analysis plan.

**10. DRUG INFORMATION**

Throughout the study, the investigational medicinal product is abraxane which will be supplied for free.

Packaging, labeling, reconstitution and storage conditions of abraxane will be detailed in the Drug Manual.

Cyclophosphamide, doxorubicin, epirubicin, 5-fluorouracil, paclitaxel, and hormonal agents are administered in accordance with their local prescribing information so these drugs are not regarded as Investigational Medicinal Products.

**10.1 Assessment of Compliance**

Treatment compliance will be monitored by drug accountability as well as the patient’s medical record and e-CRF. Missed or partial doses of study drug should be documented in
the patient’s e-CRF and also in the patient’s medical record with reasoning for the incomplete dose. Dose modifications should be also documented in the patient’s e-CRF and also in the patient’s medical record with reasoning for the dose modification.

11. BIOMARKER RESEARCH SAMPLES

The tissue and blood samples collected will be used to identify biomarkers that may be predictive of response or toxicity to the proposed chemotherapy treatments and/or prognostic for breast cancer. Since the knowledge of new markers that may correlate with disease activity and the efficacy or safety of the treatment is evolving, the analytes may change during the course of the study and may include determination of additional markers of tumorigenesis pathways and mechanisms of treatment response. The collected tumor tissue and blood samples may also be used to develop and validate diagnostic assays and allow the generation of statistically meaningful biomarker data.

Remaining sample materials after the completion of the initial biomarker assessments (e.g., aliquots of tumor RNA or DNA) may be used for further assessment of expanded marker panels.

Samples will be stored at a study’s central biological samples repository for up to 15 years after database closure, with the additional option of further long-term storage.

For sampling procedures and shipment see instructions in the appropriate sections of the Michelangelo ETNA Guidelines.

11.1 Tumor Tissue Samples

- Formalin Fixed Paraffin Embedded (FFPE) tumor block at diagnostic biopsy (mandatory) and at definite surgery (recommended).
  Submission of a tumor block from the primary tumor at diagnostic biopsy is mandatory and will be collected for study eligibility testing (regional confirmation of HER2 status, hormone receptor status and Ki67 value).
  Moreover remaining tumor tissue from the diagnostic biopsy sent to the referral pathology laboratory will be stored for additional biomarker researches, provided that the patient signed the appropriate consent.

- After the first cycle of abraxane or paclitaxel (before the start of the second cycle between days 21 and 24) an additional biopsy is recommended and the FFPE block will be sent to the study’s central biological sample repository for additional biomarker analyses (subject to patient consent)

- A FFPE tumor block from the surgical sample at the time of definite surgery is to be submitted at the study’s central biological samples repository for central pathology review and subsequent translational research. (subject to patient consent)

- Tissue collection at disease progression or recurrence (if any and subject to patient consent)
  For patients diagnosed with a local progression or recurrence or contralateral breast cancer, it is suggested the submission of an FFPE tumor block from the biopsy or surgical sample (preferred) that was used to diagnose progressive or recurrent disease, for subsequent translational research.
  Likewise for progression or recurrence in regional or distant metastases, an FFPE tumor block from the biopsy or surgery (preferred) or a fine needle aspiration (FNA) sample should be obtained and submission is recommended for future translational research.
A variety of methodologies may be applied in the above biomarker evaluation, including, but not limited to, qRT-PCR, immunohistochemistry, ELISA, in situ hybridization, and gene expression profiling. The most suitable analytical methodologies will be selected and employed. Extraction of nucleic acids (DNA and RNA) may be required to perform these analyses.

11.2 Fresh Frozen Tissue (Optional)
Sites that routinely collect fresh frozen tissue at diagnostic biopsy and at definite surgery will have the option to allocate this material, if available, to the study. This will be subject to patient consent.
A variety of methodologies may be applied in the biomarker evaluation, including, but not limited to, analysis of proteins and phospho-proteins, analyses involving RNA (gene expression, microRNA, methylation) and Next Generation Sequencing (NGS). The most suitable analytical methodologies will be selected and employed.

11.3 Collection of Whole Blood, Serum and Plasma Samples (subject to patient consent)
At baseline (prior to the start of cycle 1), after the first cycle of abraxane/paclitaxel (recommended before the start of the second cycle between day 21 and day 24) after the chemotherapy treatment (before definite surgery), and at disease progression or recurrence (if any) whole blood, serum and plasma samples (each prepared from 10 mL of peripheral blood) will be collected for biomarker assessment.

11.4 Pharmacogenetic Analysis (subject to patient consent)
Specimens for genetic-based biomarker discovery and validation will be collected from consenting patients. The pharmacogenetic information gathered through the analysis of these specimens is hoped to improve patient outcome by predicting which patients are more likely to respond to specific drug therapies, predicting which patients are susceptible to developing adverse side effects, and/or predicting which patients are likely to progress to more severe disease states. Such genetic samples collected for analysis of heritable (germline) and non-heritable (somatic) DNA variations will be double-coded: a new independent code will be added to the first code to increase confidentiality and data protection.
The results of specimen analysis from the pharmacogenetic analysis will facilitate the rational design of new pharmaceutical agents and the development of diagnostic tests, which may allow for individualized drug therapy for patients in the future.
Collection of blood samples requires patient consent. Individual patients may refuse the collection, storage and use of their blood for genetic analysis; however this will not exclude them from this study.
If the patient consents, approximately 10 mL in K3 EDTA of blood for DNA isolation will be collected. If, however, the genetic blood sample is not collected at screening/baseline or during the scheduled visit, it may be collected at any time (after randomization) during the conduct of the clinical study. For sampling procedures and shipment see instructions in the appropriate sections of the Michelangelo ETNA Guidelines.

11.5 Retention and Destruction of Samples
The specimens in the study repository will be made available for future biomarker research towards further understanding of treatment of breast cancer, related diseases and adverse events, and for the development of potential associated diagnostic assays. The implementation and use of the study repository specimens is governed by Fondazione Michelangelo and the Protocol Steering Committee, with guidance from Pathology Committee to ensure the appropriate use of the study specimens.
All biomarker specimens will be retained for new research related to this study and/or disease in accordance with the recommendations and approval of the Study Steering Committee. Samples will be only destroyed if required by local laws relating to the collection, storage and destruction of biological specimens.

**NOTE:** The specimens procured, including DNA samples, will not be used for hereditary genetic studies involving genes conferring susceptibility to cancer or other diseases. Markers examined will not be reported to the patient or her physician and will not have any bearing on her treatment.
Patients with HER2-negative, early invasive unilateral breast cancer who are at risk of disease recurrence

Informed Consent Form to participate in the

- One core biopsy block for confirmation of HER2, ER, PgR, Ki67 to be sent to Research Cooperative Group Laboratory
- Whole blood, serum and plasma samples (10 mL each) to be stored at the site and at defined timeline to be sent to the study’s central biological sample repository
- One fresh tissue sample (recommended) sample to be stored at the site and at defined timeline to be sent to the study’s central biological sample repository

RANDOMIZATION

Before the start of the second cycle between day 21 and day 24 (recommended):
- One paraffin block for biomarker analysis to be sent to the study’s central biological sample repository
- Whole blood, serum and plasma samples (10 mL each) to be stored at the site and at defined timeline to be sent to the study’s central biological sample

At surgery:
- One paraffin block for biomarker analysis to be sent to the study’s central biological sample repository
- One fresh tissue sample (recommended) to be stored at the site and at defined timeline to be sent to the study’s central biological sample repository
- Whole blood, serum and plasma samples (10 mL each) to be stored at the site and at defined timeline to be sent to the study’s central biological sample

At disease progression or recurrence (local or metastatic-IF ANY):
- One paraffin block or fine needle biopsy for biomarker analysis to be sent to the study’s central biological sample repository
- Whole blood, serum and plasma samples (10 mL each) to be stored at the site and at defined timeline to be sent to the study’s central biological sample repository

Figure 3: Study Assessment
12. SAFETY REPORTING

12.1 Monitoring, Recording and Reporting of Adverse Events

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject’s health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the CRF rather than the individual signs or symptoms of the diagnosis or syndrome.

For the purpose of this study, disease progression while on neoadjuvant chemotherapy or disease recurrence/relapse during follow-up are not to be reported as AE. It is also to be reminded that death per se is not an AE but may be the outcome of an AE.

An overdose, accidental or intentional, whether or not it is associated with an AE, or abuse, withdrawal, sensitivity or toxicity to an investigational product should be reported as an AE. If an overdose is associated with an AE, the overdose and adverse event should be reported as separate terms.

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject’s clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, or other appropriate tests and procedures.

All AEs will be recorded by the Investigator from the time the subject signs informed consent to 28 days after surgery, or 28 days after the last dose of chemotherapy if surgery is not performed for any reason. AEs and serious adverse events (SAEs) will be recorded on the AE page of the CRF and in the subject’s source documents. All SAEs must be reported to Pharmacovigilance’s Responsible within 24 hours of the Investigator’s knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form.

Beyond the above time-frame, only protocol treatment-related SAEs occurring during the follow-up part of the study are to be reported.

12.2 Evaluation of Adverse Events

A qualified Investigator will evaluate all adverse events as to:

12.2.1 Seriousness

A serious adverse event (SAE) is any AE occurring at any dose that:

- Results in death;
- Is life-threatening (i.e., in the opinion of the Investigator, the subject is at immediate risk of death from the AE);
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay);
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject’s ability to conduct normal life functions);
- Is a congenital anomaly/birth defect;
• Constitutes an important medical event.

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

Events not considered to be SAEs are hospitalizations for:

• A standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.

• Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.

• The administration of blood or platelet transfusion as routine treatment of studied indication. However, hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.

• A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.

• Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.

• A procedure that is planned (i.e., planned prior to starting of treatment on study); must be documented in the source document and the CRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.

• An elective treatment of a pre-existing condition unrelated to the studied indication.

• Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, both the AE page/screen of the CRF and the SAE Report Form must be completed.

For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to protocol drug(s) delivered, action taken regarding protocol drug(s) delivered, and outcome.

12.2.2 Severity / Intensity

For both AEs and SAEs, the Investigator must assess the severity / intensity of the event.

The severity / intensity of AEs will be graded based upon the subject’s symptoms according to the current active minor version of National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0); http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

AEs that are not defined in the NCI CTCAE should be evaluated for severity / intensity according to the following scale:

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

• Grade 2 = Moderate – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required
• **Grade 3 = Severe** – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible

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

• **Grade 5 = Death** - the event results in death

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

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

### 12.2.3 Causality

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

**Not suspected:** The temporal relationship of the adverse event to protocol drug(s) delivered administration makes a **causal relationship unlikely or remote**, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.

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

### 12.2.4 Duration

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

### 12.2.5 Action Taken

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

### 12.2.6 Outcome

The investigator will report the outcome of the event for both AEs and SAEs.

All SAEs that have not resolved upon discontinuation of the subject’s participation in the study must be followed until recovered, recovered with sequelae, not recovered (death due to another cause) or death (due to the SAE).
12.3 Abnormal Laboratory Values

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study;
- requires treatment, modification/ interruption of IP dose, or any other therapeutic intervention; or
- is judged to be of significant clinical importance.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE.

12.4 Pregnancy

12.4.1 Females of Childbearing Potential

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age) of a female subject occurring while the subject is on protocol drug(s) delivered, or within 3 months of the subject's last dose of protocol drug(s), are considered immediately reportable events. Protocol drug(s) delivered is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported in the electronic Pregnancy Reporting Form.

The Investigator will follow the female subject until completion of the pregnancy, and must notify the Sponsor immediately about the outcome of the pregnancy (either normal or abnormal outcome).

If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the protocol drug(s) should also be reported within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

12.5 Expedited Reporting of Adverse Events to Regulatory Authorities and the Ethics Committee

The Sponsor will inform relevant Regulatory Authorities and Ethics Committees;

- Of all relevant information about serious unexpected adverse events suspected to be related to the IP that are fatal or life-threatening as soon as possible, and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will be subsequently be submitted within an additional eight days.
- Of all other serious unexpected events suspected to be related to the protocol drug(s) as soon as possible, but within a maximum of fifteen days of first knowledge by the investigator.

13. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Standard Operational Procedures of the MOO at the Fondazione Michelangelo.

Accurate and reliable data collection will be assured by verification and cross-check of the e-CRFs against the investigator’s records by the study monitor (source document verification), and the maintenance of an abraxane–dispensing log by the investigator. Source document verification will be performed by monitors from the Fondazione Michelangelo or their designees including, where appropriate, monitors from other cooperative groups.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator.

14. COMMITTEES AND BOARDS

14.1 Study Committees
Three committees are planned:
- Independent Data Monitoring Committee
- Protocol Steering Committee
- Pathology Committee

14.2 Independent Data Monitoring Committee
The Independent Data Monitoring Committee (IDMC) will review safety data, including SAEs on an ongoing basis. In addition, the IDMC will review the results of the efficacy analyses. On the basis of these reviews the IDMC will make recommendations to the Protocol Steering Committee (PSC) regarding continuation, termination or modification of the study and/or the individual study treatment arms. Details of the roles, responsibilities and procedures of the IDMC will be documented by a Charter to be based on applicable international guidelines. The IDMC will operate independently of the Sponsor and participating Investigators.

14.3 Protocol Steering Committee
The Protocol Steering Committee (PSC) will be composed of country representatives and key study personnel from the Fondazione Michelangelo. The PSC will be involved in the day-to-day conduct of the trial and will not have access to unblinded efficacy data (unless or until the IDMC indicates that this is necessary). Details of the roles, responsibilities and procedures of the PSC will be documented separately. It is anticipated that the PSC will meet twice a year (or more frequently if needed) either face to face or via teleconference. Members of the PSC can attend the open sessions of the IDMC. Ad hoc members can be named as consultants to the PSC, should specific topics needed to be discussed during the meetings. The PSC reserves the rights to name specific ad hoc subcommittees if needed.
14.4 Pathology Committee
The Pathology Committee (PaC) will be composed of referral pathologists, a key person from Fondazione Michelangelo and an independent pathologist to be named by Fondazione Michelangelo. The PaC will be involved in preparing guidelines for sampling, aliquoting and storing human specimens, biohazard policy, quality control for specimens (collection and storage): Details of the roles, responsibilities and procedures of the PaC will be documented separately. It is anticipated that the PaC will meet twice a year (or more frequently if needed) either face to face or via teleconference and summaries of the meetings will be presented to the PSC.

14.5 International Advisory Board
The members of the International Advisory Board are named by Fondazione Michelangelo and have a consulting role on all breast cancer projects led by Fondazione Michelangelo. During the meetings of the Board, the status of the study and data on safety and efficacy, when available as per protocol plan, are presented and discussed.
15. ETHICAL ASPECTS

15.1 Local Regulations/Declaration of Helsinki
The investigator will ensure that this study is conducted in full conformance with the principles of the “Declaration of Helsinki” or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in “Guideline for Good Clinical Practice” ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the subject. For studies conducted in the EU/EEA countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the USA or under US IND, the investigator will additionally ensure that the basic principles of “Good Clinical Practice” as outlined in the current version of 21 CFR, subchapter D, part 312, “Responsibilities of Sponsors and Investigators”, part 50, “Protection of Human Subjects”, and part 56, “Institutional Review Boards”, are adhered to.
In other countries where “Guideline for Good Clinical Practice” exists, Michelangelo and the investigators will strictly ensure adherence to the stated provisions.

15.2 Informed Consent
It is the responsibility of the investigator, or a person designated by the investigator to obtain written informed consent from each patient participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For patients not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. After the patient and representative have orally consented to participation in the trial, the witness’s signature on the form will attest that the information in the consent form was accurately explained and understood.
The investigator or designee must also explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time, for any reason.
The e-CRFs for this study contain a section for documenting informed patient consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients, including those already being treated, should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

15.3 Independent Ethics Committees/Institutional Review Board
This protocol and any accompanying material provided to the patient (such as Patient Information Sheets used to obtain informed consent) as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval. This letter or certificate of approval will be sent by the investigator to the MOO prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.
Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.
When no local review board exists, the investigator is expected to submit the protocol to a regional committee.
16. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications will be prepared by the Fondazione Michelangelo after consultation with the PSC.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial patients, or when the change involves only logistical or administrative aspects of the trial (e.g. change in monitor or telephone numbers).

17. CONDITIONS FOR TERMINATING THE STUDY

The Fondazione Michelangelo and the PSC reserve the right to terminate the study at any time. Should this be necessary, the PSC will arrange the procedures. In terminating the study, the PSC will ensure that adequate consideration is given to the protection of the patients’ interests.

The sponsor will notify the investigators and regulatory authorities if the study is placed on administrative hold, and when the study is completed or closed to further patient enrollment.

18. STUDY DOCUMENTATION, E-CRFS AND RECORD KEEPING

18.1 Investigator's Files / Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories (1) Investigator's Study File, and (2) patient clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Patient clinical source documents include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and patient screening and enrollment logs. The investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the investigator wish to assign the study records to another party or move them to another location, the MOO must be notified in advance.

If the investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and the Fondazione Michelangelo to store these in a sealed container(s) outside the site so that they can be returned sealed to the investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside the site.
18.2 Source Documents and Background Data
The investigator shall supply the Fondazione Michelangelo on request with any required background data from the study documentation or clinic records. In case of special problems and/or governmental queries or requests for audit inspections, it is necessary to have access to the complete study records, provided that patient confidentiality is protected.

18.3 Audits and Inspections
The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Fondazione Michelangelo or its designee or to health authority inspectors after appropriate notification. The verification of the e-CRF data must be by direct inspection of source documents.

18.4 Electronic Case Report Forms
Data for this study will be captured via an Electronic Data Capture (EDC) system by using an e-CRF. The investigator should ensure the accuracy, completeness and timeliness of the data reported to the sponsor in the e-CRFs and in all required reports.

19. MONITORING THE STUDY

It is understood that the responsible Fondazione Michelangelo monitor (or other cooperative group monitor, or designee) will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial (e-CRFs and other pertinent data) provided that patient confidentiality is maintained in accordance with local requirements.

It will be the monitor's responsibility to inspect the e-CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the e-CRF. The investigator (or his/her deputy) agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

20. CONFIDENTIALITY OF TRIAL DOCUMENTS AND PATIENT RECORDS

The investigator must assure that patients’ anonymity will be maintained and that their identities are protected from unauthorized parties. On e-CRFs or other documents submitted to the MOO or the Central Office of your Cooperative Research Group, patients should not be identified by their names, but by an identification code. The investigator should keep a patient enrollment log showing codes, names and addresses. The investigator should maintain documents not for submission to the MOO or the Central Office of your Cooperative Research Group, e.g., patients’ written consent forms, in strict confidence.
21. PUBLICATION OF DATA

The results of this study may be published or presented at scientific meetings. In accordance with standard practice, this large multicenter trial will be published in its entirety and not as individual center data. Additional publications based on the exploratory research aspects of the trial are expected to be published after the main study data are made public. Details of publication policy, including authorship of key papers, will be decided by the PSC. All papers and presentations which include data from the study should be submitted to the PSC for approval before being made public.

21.1 Right to Publication

As a sponsor of the study, Fondazione Michelangelo grants that participating investigators have full rights to publish and present the results of the study.
### Table 10: Schedule of Assessments: Screening, Treatment Period and Follow-up

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<th>End of Study **</th>
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**Notes:**
- X: Assessment performed.
- [X]: Assessment performed continuously.

---

**ETNA – Protocol Version 2.0 - 10th March 2014**
**Legend:**

1. Height, weight, BSA, ECOG performance status, vital signs (blood pressure).
2. Hemoglobin (Hb), white blood count (WBC) with differential, platelet count.
3. Serum creatinine, electrolytes (sodium, magnesium, potassium, calcium), lactate dehydrogenase (L-DHL), total bilirubin, AST/SGOT, ALT/SGPT, alkaline phosphatase (ALP).
4. Mandatory if ALP is >ULN or if patient has unexplained bone pain.
5. Electrocardiograph (ECG), Left ventricular ejection fraction (LVEF) by echocardiography or multi-gated scintigraphic scan (MUGA scan).
6. A negative serum pregnancy test must be provided within 72 hours prior starting study.
7. Weight, ECOG performance status.
8. Hemoglobin (Hb), white blood count (WBC) with neutrophils and platelet count.
10. Liver imaging is mandatory in patients with AST or alkaline phosphatase > ULN.
11. Adverse events (AEs) and serious adverse events (SAEs) must be collected continuously from the informed consent signature date to 28 days from the date of last chemotherapy administration.

° Screening evaluations are accepted within 3 weeks before registration.

oo If clips or other injectable materials were not placed at core biopsy, it is recommended that during physical examination (at the screening or before starting neoadjuvant chemotherapy), while the patient lies on her back, the center of the palpable lesion in the primary tumor be marked by a tattoo (extreme of the two largest perpendicular diameters). Exception: according to local guidelines, identification of the primary tumor area is allowed before the second cycle of either taxane.

§ Exams required at cycle 1, before starting chemotherapy, are accepted if performed within 4 weeks from registration except for hematological assessment that must be performed within 2 weeks (15 days).

* Within 3 weeks from the date of last chemotherapy administration.

** At 4-5 weeks (28-35 days) from the date of surgery.

[] Not Mandatory: suggested in accordance with Institutional Standards.
22. REFERENCES


25. Rugo HS, Barry WT, Moreno-Aspitia A, et al. CALGB 40502/NCCTG N063H: Randomized phase III trial of weekly paclitaxel (p) compared to nanoparticle albumin-bound nab-paclitaxel (NP) or ixabepilone (Ix) with or without bevacizumab (B) as first-line therapy for locally recurrent or metastatic breast cancer (MBC). J Clin Oncol 2012; 30 (suppl., abstract CRA1002).


ETNA STUDY (FM12B01)

Neoadjuvant chemotherapy with nab-paclitaxel in women with HER2-negative high-risk breast cancer

STATISTICAL ANALYSIS PLAN

<table>
<thead>
<tr>
<th>Name of Sponsor</th>
<th>Fondazione Michelangelo</th>
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<td>Phase III</td>
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<tr>
<td>Version Number</td>
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# Revision History

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<th>Date of Approval</th>
<th>Reason for Change</th>
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<td>1.0</td>
<td>Domenico Magazzù</td>
<td>23/01/2014</td>
<td>First release of the document</td>
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| 2.0     | Domenico Magazzù  | 09/09/2015       | - Added a new analysis population: patients treated with combination chemotherapy  
- Included in the PP population patients who did not complete chemotherapy because of disease progression  
- Added disease stage or tumor subtype not meeting the inclusion criteria as reason for exclusion from PP population  
- Included method for handling with missing axillary evaluation at surgery with regard to the analysis of primary endpoint  
- Added study-specific conventions for the evaluation of the clinical response  
- Rephrased the paragraphs 11.2.3 ‘EFS’ and 11.2.3.1 ‘Relationship between pCR and EFS’  
- Clarified the timing of analysis of time to event endpoints and of release of the final study report  
- Changed the algorithm for the computing the cumulative dose for combination chemotherapy  
- Specified that only version 16.0 of MedDRA dictionary will be used for coding  
- Clarified the analysis of laboratory parameters  
- Changed some table titles |
| 3.0     | Raffaella De Fato | 11/02/2016       | - Patient enrollment considerations  
- pCR consideration update. During the IDMC review meetings, the description of pCR was performed and presented coded by treatment arm (Arm A vs Arm B). For this reason, the OR computation direction will not be changed in the final analysis |
even if the arm correspondence is Paclitaxel (arm A) vs Abraxane (arm B). In this way, the OR corresponding to an absolute difference of 10% expected should have been 0.58.

- RBC analysis performants if 25% data available
- Specified that only the version of MedDRA dictionary available at cut-off date will be used for coding
- Update in appendix 1 table list
Signature Page

Version number: 3.0

Prepared by

Raffaella De Fato
Study Biostatistician

Date

Approved by

Pinuccia Valagussa
Sponsor Delegate

Date

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1. **INTRODUCTION**

This document describes in detail the descriptive and statistical analyses of the ETNA study which are to be performed for the preparation of the clinical study report. All analyses take origin from the specifications in the statistical section of the protocol.

2. **STUDY RATIONALE**

Neoadjuvant chemotherapy is an established approach for managing patients with large early stage breast cancer and with locally advanced disease, and provides an opportunity for collection of specimens for correlative science studies to identify predictive markers for response to specific agents [1].

The rationale for evaluating preoperative chemotherapy in patients with non-metastatic breast cancer was provided by hypotheses formulated from findings obtained in laboratory investigations and from results of preoperative chemotherapy for early stage and locally advanced cancers. The presence of clinically and pathologically assessable disease allows for assessing the response to the administered neoadjuvant regimen and provides a unique opportunity to evaluate pretreatment specimens for predictive markers.

To potentially avoid overtreatment in patients with hormonal receptor positive tumors, in our study we will use the above mentioned classification to select HER2 negative high risk tumors to be treated, namely Luminal B and triple negative categories.

Due to the success of available therapies, the conduct of adjuvant studies requires ever larger numbers of patients and long periods of follow up to assess the value of new therapies [2]. In recent years the neoadjuvant approach is gaining increasing consideration as a tool for the rapid scrutiny and ranking of new and established therapies, especially since the use and application in groups of patients selected for homogeneous molecular characteristics.

The overall concept of the approach and the overall experimental design is in two phases as illustrated in the following scheme:

---

**Overall Design**

**Part 1**
- Will test in a neoadjuvant setting the presence of biomarkers associated with response, resistance and clinical/pathological endpoints

**Part 2**
- Will validate two concepts:
  a) applicability of markers and their clinical utility in the same neoadjuvant setting
  b) more general applicability to the adjuvant scenario
**Figure 1: Overall Design**

In the present study the neoadjuvant approach will be used to compare the complete pathological response rate of two regimens: a) weekly abraxane (Abraxane®, abraxane) followed by AC or EC or FEC before surgery; b) weekly paclitaxel followed by AC or EC or FEC before surgery.

In our study we will use hormonal receptor status and Ki67 values to select HER2 negative tumors at high risk of disease relapse, namely Luminal B and triple negative categories.

Also, several IHC and molecular assays will be performed before and during the period of chemotherapy administration and at surgery with the goal of defining a marker of efficacy to be later validated in a larger adjuvant setting.

### 2.1 JUSTIFICATION FOR THE USE OF PATHOLOGICAL COMPLETE RESPONSE AS PRIMARY ENDPOINT

Randomized neoadjuvant trials suggest that a pathologic complete response (pCR) may predict disease-free survival or overall survival among patients with breast cancer who are treated with preoperative systemic therapy [3-7]. If higher pCR rates obtained with more effective regimens continue to predict for improved outcome, then pCR could be used as an intermediate endpoint in testing new chemotherapy regimens as well as newer targeted therapies [8].

For example, in a neoadjuvant trial of chemotherapy with or without trastuzumab in locally advanced breast cancer [3], the group that received trastuzumab had a near doubling of the pCR rate (38% vs 19%, p = 0.001) that translated into a statistically significant 3-year disease-free survival rate of 71% vs 50% (p = 0.013).

### 3. STUDY DESIGN

This is an open-label, randomized phase III trial (Fig. 1).

Patients will be randomized to one of the 2 possible treatment arms in a 1:1 ratio (Arm A, abraxane/Arm B, paclitaxel):

**Arm A**

*Abraxane* at the dosage of 125 mg/m² over 30 minutes given week 1, 2 and 3 followed by 1 week rest to be repeated for 4 cycles followed by

*AC* or *EC* (doxorubicin or epirubicin and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

or

*FEC* (fluorouracil, epirubicin, and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

**Arm B**

*Paclitaxel* at the dosage of 90 mg/m² diluted in 250 mL of WFI over 1 hour given week 1, 2 and 3 followed by 1 week rest to be repeated for 4 cycles followed by

*AC* or *EC* (adriamycin or epirubicin and cyclophosphamide) on day 1 every 3 weeks for 4 cycles

or

Page 8 of 30
**FEC** (fluorouracil, epirubicin, and cyclophosphamide) on day 1 every three weeks for 4 cycles

No neoadjuvant endocrine therapy is allowed in patients with hormone receptor positive disease (ER and/or PgR-positive).

The stratification variables will be:

a) Cooperative Research Group

b) Disease stage [operable (tumor stage: T2N0-1; T3N0) and locally advanced (T3N1; T4 any N, any T N2-3)]

c) Tumor subtype [luminal B intermediate (HER2 negative, ER or PGR positive, Ki67 from 14% to 20%) vs luminal B high (HER2 negative, ER or PGR positive, Ki67 >20%) vs triple negative tumors (HER2 negative, ER negative and Pgr negative, Ki67 any value)]

![Study Design Flow-Chart](https://jamanetwork.com/)

*Figure 2: Study Design Flow-Chart*
4. STUDY OBJECTIVES

4.1 PRIMARY OBJECTIVE

- To compare the rate of pathologic Complete Response (pCR, defined as ypT0-Tis, ypN0) for abraxane vs paclitaxel

4.2 SECONDARY OBJECTIVES

- To compare the pCR rates in the two main subgroups of ER and/or PgR positive tumors and triple-negative tumors separately
- To compare the rate of clinical overall response (cOR) after the first 4 cycles of abraxane vs paclitaxel
- To compare the rate of cOR after the entire preoperative chemotherapy (i.e. before surgery) in the study arms of abraxane vs paclitaxel
- To compare the Event Free Survival (EFS, i.e. disease progression while on primary therapy or disease recurrence after surgery) in the study arms of abraxane vs paclitaxel
- To compare the Distant EFS (DEFS) in the study arms of abraxane vs paclitaxel
- To compare the Local EFS (LEFS) in the two study arms of abraxane and paclitaxel
- To compare the Regional EFS (REFS) in the two study arms of abraxane and paclitaxel
- To compare the overall survival (OS) in the study arms of abraxane vs paclitaxel
- To evaluate the tolerability of the treatment regimens in the different study arms

- To conduct molecular and clinical analyses to assess the presence of predictive markers of benefit

5. SAMPLE SIZE CONSIDERATIONS

The primary endpoint is to compare the rate of pathologic Complete Response (pCR) between abraxane and paclitaxel.

Treatment contrast is expressed in terms of relative measure of effect, i.e. an odds ratio (OR) equal to 1.7, corresponding to an absolute difference of 10% when the pCR for paclitaxel is 20%, setting a target pCR for abraxane to 30%.

The derived OR was then used to employ a two sided Cochran-Mantel-Haenszel design, stratifying the comparison between the 2 arms by breast cancer phenotype (triple negative, others).

Assuming to screen 40% patients triple negative and 60% patients with other tumors, with an estimated response rate to paclitaxel of 32% and 15%, respectively, 632 patients (316 per arm) are required to reject the OR set by the null hypothesis of 1 when the OR is actually 1.7, with power=80% and the false positive rate=5%.

The expected overall pathological complete response rate in breast and axilla (pCR) for HER2-patients with operable and locally advanced breast cancer, that is 20%, is taken from results of the GeparTrio study [9] where patients were essentially treated with a docetaxel containing regimen. In the GeparTrio trial, in a sample of about 1000 HER2-patients, a pCR at surgery was recorded for 20% of them. The recent updated analysis presented at the San Antonio Breast
Cancer Conference [10] reported a pCR for 133 patients out of 362 triple negative patients (36.7%) and for 39 patients out of 211 luminal B patients (18.5%).

Estimates of paclitaxel effect in the 2 study sub-populations (triple negative vs other) are based on the NOAH study in locally advanced breast cancers [5]. In the NOAH, in the limited parallel group of HER2-negative tumors 11 patients out of 35 triple negative patients (31%) and 2 patients out of 16 in the other tumors (12.5%) attained a pCR.

Because of the above mentioned statistical considerations, enrolment to either of the two tumor subtypes will be discontinued after reaching the estimated percentage and will be continued only on the other subtype to reach a total of 632 patients.

Sample size computations were made using Pass 2008 software.

5.1 PATIENT ENROLLEMENT

At the end of the patient enrollment, a consideration was performed: the proportion assumed for the subtype of tumors (40% triple negative patients and 60% patients with other tumors) was not reproduced. The main reason is that the assumption was performed considering the FM studies where the definition of triple negative tumor was performed considering the value of ER and PgR minor of 10%. Instead, during the ETNA study, the classification of tumor subtype was performed considering 1% as limit value for negativity/positivity.

When the percentage of Luminal B patients enrollment was reached, the Michelangelo team required to IDMC if the enrollment should be stopped for this tumor subtype, and, attending this decision, the randomization proceeded for all patients contacted in the meantime.

For these reasons, at the end of enrollment, more patients than requested was randomized (695 patient randomized vs 632 planned) and the percentage of triple negative patient randomized is 31.5%.

6. INTERIM ANALYSES AND INDEPENDENT DATA MONITORING COMMITTEE (IDMC)

The safety and tolerability profile of the study drugs will be monitored on regular basis by an Independent Data Monitoring Committee (IDMC). Timing, organization and responsibilities of the meetings as well as the structure and content of the review material are detailed in a separate charter.

The ETNA protocol has no planned interim efficacy analyses (either for superiority or futility) for the primary endpoint of the achievement of pathological complete remission (pCR, defined as ypT0-Tis, ypN0). In order to allow the IDMC to assess the risk/benefit ratio of the protocol treatments, data on clinical disease progression while on neoadjuvant treatment will be made available rather than pCR data.

Briefly, the first review meeting is scheduled after 50 subjects have completed the first 4 cycles of either taxane. Subsequent meetings to review data will occur approximately every 6 months thereafter. After each review, the IDMC may suggest modification to the trial protocol. These modifications may include, but are not limited to:

- Changes in inclusion/exclusion criteria
- Frequency of visits or safety monitoring
- Modifications in study drugs dosage and/or schedule
• Alterations in trial procedures or trial conduct
• Changes to the statistical analysis plan
• Continuation of the trial according to the protocol and any relevant amendments
• Discontinuation of the trial (with provisions for orderly discontinuation in accordance with good clinical practice).

A decision on the study conduct based on the IDMC’s findings will then be made by the Study Sponsor (Fondazione Michelangelo) and the Chair and members of the Protocol Steering Committee.

Most of the tables described in this plan can fit well IDCM requirements; in particular patient disposition, demography, baseline and tumor characteristics as well as all the safety tables will be also produced for the IDCM periodic meetings with masked treatment assignment (e.g. A and B), in order to ensure as high objectivity as possible in the evaluation of study conduct and safety by the IDMC members.

7. ANALYSIS POPULATIONS

Intent-to-Treat Population (ITT)
The primary population for all efficacy endpoints will be the ITT (intent-to-treat) population, defined as all randomized patients classified on the basis of the treatment arm they were randomized to.

Per Protocol Population (PP)
The PP population is defined as all randomized patients who completed the scheduled courses of treatment unless they progressed during the chemotherapy, in which case they will be considered eligible for the PP population, and without any major protocol violation regarding the efficacy evaluation and any other reason for exclusion from efficacy analysis.

Specifically, the detection of any of the following items will exclude the patient from the PP population:
• Evidence of bilateral invasive breast cancer or metastatic disease (M1)
• Disease stage or tumor subtype not fulfilling the study inclusion criteria
• Any previous therapy for breast cancer, including chemotherapy, hormonal therapy and radiotherapy
• Hematological and/or biochemical values not meeting the eligibility criteria, as specified in the exclusion criteria section
• Missing evaluation of pathological complete response at surgery

The primary endpoint and the key secondary endpoint, i.e. event free survival and distant event free survival, will be also analyzed in the PP population, integrating the results observed in the primary population.

Safety Population
The safety population is defined as all randomized patients who received at least one dose of either abraxane or paclitaxel. Patients will be classified according to the treatment administered, regardless of any discrepancy with respect to the treatment they were randomized to. The safety population will be used for all safety analyses.

Patients treated with combination chemotherapy
It is formed of all patients included in the safety population who received at least one cycle of the combination chemotherapy. This population will be used to describe treatment exposure and dose modifications concerning the combination chemotherapy and for the tabulation of adverse event related to or occurred during the combination chemotherapy. It may differ from the safety population if some patients who received the taxane therapy did not receive the combination, due to investigator’s decision or patient’s refusal to continue therapy.

8. GENERAL CONSIDERATIONS FOR STATISTICAL ANALYSIS AND REPORTING

All tables will be presented broken down by treatment arm; patient disposition, demography, baseline and tumor characteristics will be also summarized overall, i.e. not taking into account the arm which the patient was randomized to. According to the ICH guideline E3 – Structure and Contents of Clinical Study Report – all the tables produced will form the appendix 14 of the clinical study report, patient data listings will constitute the appendix 16.2. Any output deriving from the application of inferential tests and procedures will make part of appendix 16.1.9 (Documentation of Statistical Methods).

All inferential analyses will be conducted at two-sided 5% significance level if not otherwise specified. The analysis will be conducted by using SAS version 9.1 or higher. All variables collected in the CRF will be displayed in patient data listing, by the relevant form, for all randomized patients or for relevant subsets as specified in the specific sections below. These listings may also contain some derived variables as needed, in order to support the interpretation of the corresponding tables. As a general rule, the outputs will be sorted by centre, patient ID, visit and date of assessment.

9. PATIENT DISPOSITION

Descriptive statistics (number and percentage of patients) will be provided for

- The number of screened (registered) and randomized patients overall and by country and site.
- Randomization status by stratification variables (research cooperative group, disease stage and tumor subtype)
- Analysis populations
- Patients who completed and discontinued the study treatment along with reasons for discontinuation, patients still on treatment at the time of analysis.
- Patients entering the follow-up phase, patients who terminated the follow-up phase along with reasons (death, consent withdrawal, lost to follow up), patients still in follow-up.
- Cumulative number of progressions/recurrences of any type and by type, cumulative number of deaths overall and according to progression/recurrence status (with/without).
- Major protocol violations and reason for exclusion from any analysis as defined in the per-protocol population section

The follow-up phase begins when the patient undergoes surgery, therefore if no data are reported in the surgery form the patient will be only analyzed for the treatment portion of the study.
10. **DEMOGRAPHY AND BASELINE CHARACTERISTICS**

The analysis of demographic and baseline characteristics will be carried out in both the ITT and PP population.

10.1 **DEMOGRAPHY AND PATIENT CHARACTERISTICS**

Appropriate statistics will be calculated for age (years), age category (<40, 40-55, >55), ethnic group, weight, height, BSA, ECOG performance status, systolic and diastolic blood pressure. Age will be calculated at the date of randomization, using date of birth information.

10.2 **TUMOR CHARACTERISTICS**

Tumor characteristics will be reported according to two groupings: disease stage (evaluated locally) and information from the centralized core biopsy characterizing the tumor subtype.

10.2.1 **Disease stage**

The following variable will be analyzed: histology (ductal invasive, lobular invasive, other), TNM staging, T stage alone, lymph node status (N0, N1, N2, N3), median tumor size, disease stage (operable, locally advanced), tumor grade (well, moderately and poorly differentiated).

10.2.2 **Tumor Subtype**

As far as the data defining the tumor subtype are concerned, only information from the centralized assessment will be tabulated, since these are the ones determining eligibility and subsequent centralized randomization of the patients. Data from local evaluation will be presented in listings.

Descriptive statistics will be presented for hormone receptor status (ER positive and/or PR positive, ER and PR both negative), Ki67, analyzed as both continuous variable and according to the following categories 14-20%, >20%), tumor subtype (luminal B overall/intermediate/high, triple negative).

10.3 **MEDICAL AND CARDIAC HISTORY**

Medical history will be coded by MedDRA dictionary and described by System Organ Class and Preferred Term; Cardiac history will be analyzed according to the CRF preprinted categories.

11. **EFFICACY**

All efficacy endpoints will be analyzed in the ITT population. The primary efficacy endpoint, the rate of pCR, and the key secondary efficacy endpoints, the event free survival and the distant event free survival, will be additionally analyzed in the PP population. Results will be compared for consistency.

11.1 **PRIMARY EFFICACY ENDPOINT: RATE OF PATHOLOGICAL COMPLETE RESPONSE (PCR)**

The primary endpoint is to compare the rate of pCR at surgery between abraxane and paclitaxel containing regimens.

Pathological complete response is defined as absence of invasive disease in breast and nodes (ypT0/ypTis, ypN0). From a practical point of view, patients will be counted as pathological responders if in the surgery form the box ‘absence of invasive cells’ is marked and the number
of positive lymphnodes is 0. In this study axillary dissection at surgery may not be repeated if the patient was clinically N0 and with a negative sentinel lymphnode at screening. Under these circumstances, patients with missing axillary evaluation at surgery will be considered ypN0. In all other cases, the absence of axillary assessment at surgery will determine patients be classified as non-responder for the primary endpoint. This issue was discussed also during the IDMC meeting and confirmed by the board.

Absolute and relative frequencies of patients presenting valid data for the primary endpoint will be provided.

The primary variable will be categorized in responders and non-responders patients, with no separate level for missing/unknown values. The way missing values will be handled is detailed in the next paragraph. Exact 95% confidence intervals (Clopper-Pearson method) for the pCR rate will be provided. The denominator will be the ITT population within each arm.

The comparison among treatments will be carried out using a two-sided Cochran-Mantel-Haenszel (CMH) test, controlling for tumor sub-group (triple negative and the 2 luminal B categories pooled together) and disease stage (operable vs. locally advanced). A composite variable will be created and patients will be classified in one of the following four categories: triple negative non-locally advanced, triple negative locally advanced, luminal B non-locally advanced, luminal B locally advanced. This variable will be employed in the CMH test.

Assuming no interaction between treatments and the stratification factors, the pooled effect will be estimated by a unique CMH odds ratio (OR) and its 95% confidence interval; the corresponding p-value will be also reported. Should the Breslow-Day test for homogeneity of the OR across strata turn out to be significant, ORs will be also presented within each level of the stratification variable along with 95% confidence intervals, with the meaning that an interaction is present.

Treatment effect will be also expressed as crude (unadjusted) absolute difference in pCR rates between treatments. 95% confidence intervals will be calculated according to the Wald method.

As sensitivity analysis, a multivariate analysis to estimate the net effect of treatment adjusted for all stratification factors (research cooperative group, disease stage, tumor subtype), age (<=50, >50) and type of combination chemotherapy will be performed by a logistic regression model.

11.1.1 Subgroup Analysis

Sub-group analysis will be conducted within each level of stratification factors (i.e. cooperative research group, tumor subtype, disease stage), within each combination therapy (AC, EC, FEC) and within age categories (<=50, >50); crude absolute difference in pCR rates, crude ORs and 95% confidence intervals will be reported. As for combination therapies and age group, treatment effect will be controlled for the four levels of disease stage and tumor subgroup combined together, in order to avoid bias due to potential imbalances of stratification factors across arms; adjusted CMH ORs will be shown.

Differences in the pCR rates between treatment arms will be tested by either a chi-square test or a CMH test (the latter for combination therapy and age categories).

Homogeneity of treatment effect across each subgroup variable levels will be tested through the addition of an interaction term treatment*subgroup variable in a logistic regression model, containing treatment and the relevant subgroup variable as predictors (and adjusted for disease
stage and tumor subgroup with regard to the analysis by combination therapy and age). The Wald test will be used to assess homogeneity.

11.1.2 Handling of missing values
Patients who drop out of the study before surgery will be included in the primary analysis, following the ITT principle, and considered as non-responder as well as randomized patients who are never treated, whatever is the reason for not receiving the treatment. Patients with missing or partial evaluation of pathological complete response at surgery will be included in the primary analysis and analyzed as non-responder.

A sensitivity exploratory analysis will be carried out using the worst/best case approach. This method discriminate between the pattern of missing values in the experimental arm, in which they will be replaced with ‘non-responder’ status, and the pattern in the control arm in which missing values will be replaced with ‘responder status’. Results from this approach will be compared to that of the primary analysis for consistency: if the estimated treatment effect is not affected by the way missing values are handled, the conclusions and the external validity of the trial will be stronger.

11.1.3 Surgery data
To integrate the results from the primary analysis, the information collected in the surgery form of the eCRF will be analyzed; frequency distributions will be provided for: type of surgery, axillary dissection, histopathologic type, histologic grade, in-situ cancer lesions and pTNM. The other data will be displayed in listings.

11.1.4 Residual cancer tumor bed characteristics
In the ETNA study, data on residual cancer tumor bed characteristics are collected, though not mandatory. If the amount of missing values is negligible, exploratory analysis will address the prognostic value of the residual cancer burden (RCB), calculated according to the formula reported in Symmans et al. [11]. RCB measurement provides a continuous parameter of response that may add insights in the context of pCR. The cut-off points of 1.36 and 3.28 (as per Symmans’ paper) will be used to discriminate among 4 classes with potential increasing poor prognosis: RCB-0 (RCB =0), RCB-I (0 < RCB <=1.36), RCB-II (1.36 < RCB <= 3.28) and RCB-III (RCB > 3.28).

If at least of 25% of patients have data available for the RCB computation, the following analyses will be performed:

a) Frequency tables of patients achieving RCB-0 + RCB-I. Differences among treatments will be tested in the same fashion as for the primary endpoint

b) Summary statistics of RCB as continuous variable in patients not achieving a pCR. Differences between treatments will be tested by a Wilcoxon rank-sum test.

c) Frequency tables of patients by RCB classes from I to III in patients not achieving a pCR. Differences between treatments will be tested by a chi-square test; ORs and 95% CI (Abraxane vs. Paclitaxel) for the two comparisons RBC-II and RBC-III vs. the reference category RBC-I will be provided.

11.2 SECONDARY EFFICACY ENDPOINT
11.2.1 **pCR rate in the two main subgroups of ER and/or PgR positive tumors and triple-negative tumors**

pCR rate will be analyzed separately in the two main tumor subtypes as part of the subgroup analyses for the primary endpoint as stated above. Due to the exploratory nature of this endpoint, no alpha error adjustment for multiple testing will be made.

11.2.2 **Clinical Overall Response (cOR) rate**

For the purpose of this study, all clinical measurements will be assessed by palpation of the breast and regional nodes. The objective response rate, defined as the proportion of patients who attained either a clinical complete response (cCR) or a partial response (cPR) during the study, will be evaluated according to RECIST criteria (v 1.1), adapted to this protocol and detailed here following.

Clinical response will be assessed after either taxane therapy and at the end of the entire neoadjuvant treatment.

Patients eligible for the ETNA protocol must present with either of the following categories:
- Non locally advanced breast cancer (T2N0-1; T3N0)
- Locally advanced breast cancer (T3N1; T4 any N; any T N2-3)

**Before starting neoadjuvant therapy** the following considerations are taken into account

- **Breast lesions**
  - T2 and T3 lesions can be fully measured by palpation and their longest diameter is reported in the CRF: they are considered target lesions
  - T4 lesions are generally measurable by palpation if they are T4a, T4b, T4c and their longest diameter is reported in the CRF: they are considered target lesions. Exceptions may be patients whose entire breast is involved (e.g. lesion takes up the whole breast): they are considered non-target lesions
  - T4d lesions are not measurable. T4d is considered inflammatory carcinoma, a presentation where a nodule is not palpable and the overlying skin is red, swollen and painful to the touch. T4d lesions are considered non-target lesions

- **Nodal involvement**
  - N0: no cancer nodal involvement is found in the ipsilateral axilla
  - N1: at palpation ipsilateral axillary nodes are not stuck to the surrounding tissues and the presence of cancer is only assumed. Of note, lymph nodes are anatomical structures that can be palpated anyhow: the CRF does not require they to be measured and they are not to be considered target lesions regardless of their dimensions
  - N2: at palpation ipsilateral axillary nodes are felt and they are stuck and can be measured. They are considered as target lesions and their longest diameter is reported in the CRF.
  - N3: they may be located above or below the collarbone (supraclavicular of infraclavicular nodes) and are clinical palpable or may be located at the internal mammary chain in which case they can be detected only radiologically and are
regarded as non-target lesions. When measurable (target lesions), the largest
dimension of supraclavicular and infraclavicular nodes is reported in the CRF.

At the time of the on-treatment assessments, after either taxane and at the end of the protocol
chemotherapy, response levels are not directly given by the investigators but will be assessed
according to the RECIST criteria, based on the measurements reported in the CRF for
measurable lesions.

The following conventions will be followed:

- For breast lesions and N2/N3 lesions which are measurable at the start of chemotherapy
  and are no more measurable at the above assessments and the investigators have not
  reported a value = 0 at the longest diameters
  - the reason for non-measurability is either ‘complete response’ or ‘clinical complete
    remission’ the longest diameter will be considered to be = 0
  - the reason for non-measurability is ‘not palpable’ a query will be issued and if the
    investigator consider it is a clinical complete remission, he/she will be asked to
    insert the value of 0 at the longest diameter
  - the reason for non-measurability is ‘too small to measure’ a default value of 5 mm
    will be assigned
- For breast lesions which are not measurable at the start of chemotherapy, especially for
  very large lesions involving the whole breast and for inflammatory cancers, the data will be
  presented to a clinical reviewer and his/her judgment will be taken into account.

Response Criteria at each evaluation
The following criteria will be employed at the completion of the assigned taxane regimen (before
starting the anthracycline therapy) and at the conclusion of the entire neoadjuvant therapy
(before surgery).

Clinical Complete Response (cCR)
Disappearance of all target and non-target lesions identified at baseline, with no evidence of
disease progression.

Clinical Partial Response (cPR)
At least a 30% decrease in the sum of the longest diameter (LD) of target lesions (taking as
reference the baseline sum of the longest diameter). No definite progression of non-target
lesions. No evidence of new lesions.

Clinical Stable Disease (cSD)
Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for
progressive disease.

Progressive Disease (PD)
- Sum of breast lesions and N2/N3 diameters increasing at the assessment:
  - If the increase meets the RECIST criteria of at least 20% increase over the nadir but
    is less than 23% and the investigator has not checked ‘progressive disease’ in the
    appropriate CRF form, the investigator’s assessment prevails. The reasons for this
    are mainly due to the fact that the clinical palpation may not be precise as a caliper
measurement or to the fact that the patient was lying differently in the two different assessments

- If the increase does not meet the RECIST criteria of at least 20% increase but the investigator checked ‘progressive disease’ in the appropriate CRF, a query will be issued but the investigator’s assessment prevails.

Other manifestations of progressive disease would also be classified as disease progression, e.g.: appearance of one or more new lesions in the breast, regional lymph nodes or distant sites; unequivocal progression of existing non-target lesions; appearance of inflammatory carcinoma on clinical exam.

**Clinical Overall Response (cOR) rate**

The clinical overall response (cOR) rate is defined as the proportion of patients who attained either a clinical complete response (cCR) or a partial response (cPR) at two specific timepoints: end of taxane period, end of the combination chemotherapy. No confirmation of response is required.

The number and percentage of patients achieving cCR and cPR will be reported separately and overall as cOR.

Treatment comparison on cOR will be carried out by Cochran-Mantel-Haenszel test, using tumor sub-group and disease stage as stratification factors, combined together as defined for the primary endpoint; exact 95% confidence intervals for cOR will be reported within each arm. The strength of the association between treatment and outcome of interest will be measured by both crude absolute difference in response rates and CMH OR, with 95% confidence intervals.

11.2.3 **Event Free Survival (EFS)**

For the purpose of this protocol the EFS is defined as the time from randomization to the first date of disease progression while on primary therapy or disease recurrence (local, regional, distant, invasive contralateral breast) after surgery or death due to any cause. Patients who at the time of the analysis do not present any of the above events will be censored at the maximum date between a) the date of last visit on follow-up b) last date of imaging tumor assessment before surgery c) last treatment date d) the date of randomization. If follow up data are reported but a) is not available, it will be replaced by the date of last contact.

EFS will be analyzed by the Kaplan-Meier (KM) method: quartiles of EFS along with 95% CI, estimates at fixed time points (e.g. 2 and 4 years) and KM curves will be provided. Differences in the EFS distribution between abraxane and paclitaxel will be tested by the log-rank test, stratified by tumor subgroup and disease stage. Hazard ratio and its 95% CI will be derived from Cox regression stratified by tumor subgroup and disease stage.

Subgroup analysis will be performed based on the same strata as for the primary endpoint with the addition of the two categories as defined by the primary endpoint status (pCR/no pCR). Crude HRs and 95% confidence intervals will be reported by means of Cox regression model using treatment as the only predictor, with the exception of combination therapies, age group and pCR status, for which treatment effect will be controlled for disease stage and tumor subgroup, in order to avoid bias due to potential imbalances of stratification factors across arms. Homogeneity of treatment effect will be tested through the addition of the interaction term treatment*subgroup variable in a Cox model containing treatment and the relevant subgroup.
variable as predictors (stratified by disease stage and tumor subtype, for combination therapies, age group and pCR status).

11.2.3.1 Relationship between pCR and EFS
The prognostic value of pCR on EFS will be evaluated by a univariate Cox model with EFS as response variable and pCR as independent variable, stratified by tumor subgroup and disease stage.

The predictive value of pCR for a treatment effect on EFS (i.e. evaluating whether achievement of a pCR predicts a stronger benefit from treatment with Abraxane on long term outcome than does the non-pCR category) will be evaluated as part of the above mentioned subgroup analysis. The potential surrogacy of pCR for EFS will be explored by a Cox model stratified by disease stage and tumor subgroup, including pCR status and treatment as predictors and EFS as response variable. Assuming a statistically significant treatment effect on EFS and on pCR, and in turn a statistically significant pCR effect on EFS, the fourth (and probably the most important) Prentice criteria to support surrogacy [12-13] requires that pCR retain its statistical significance whereas treatment effect becomes no longer significant with the hazard ratio shifting very close to 1, when both variables are present in the Cox model. Homogeneity of treatment effect across pCR status levels will be tested by an appropriate interaction term.

11.2.4 Distant Event Free Survival (DEFS)
DEFS is defined as the time from randomization to the first date of distant progression while on primary therapy or distant recurrence (invasive contralateral breast included) after surgery or death due to any cause.

The analysis will be carried out by the competing risk method, considering loco-regional progression/recurrences as competing events. Patients experiencing the event of interest will be given a censoring code of 1. Patients experiencing locoregional events will not be censored as in the usual Kaplan-Maier approach but will be assigned a censoring code of 2. Real censored patients, i.e. those who did not experience any type of event will be given a censoring code of 0. In the latter case, the date of censoring will be derived using the same algorithm as for EFS. For patients experiencing both the event of interest and the competing event, the one occurring first will be counted.

Cumulative incidence functions will be used to generate DEFS curves, quartiles and estimates at fixed time points such as 2 and 4 years together with 95% CI will be reported.

Differences in cumulative incidence functions between abraxane and paclitaxel will be tested by the Gray’s test. Hazard ratios and 95% CI will be obtained by a proportional subdistribution hazard model as proposed by Fine and Gray, adjusting for tumor subgroup and disease stage.

11.2.5 Time to Local Event (TTLE)
TTLE is defined as the time from randomization to the first date of local progression while on primary therapy or regional recurrence after surgery.

The method of analysis will be the same as for DEFS, with the difference that local events will be the event of interest while distant and regional events and deaths will be regarded as competing events.

11.2.6 Time to Regional Event (TTRE)
TTRE is defined as the time from randomization to the first date of regional progression while on primary therapy or regional recurrence after surgery.
The method of analysis will be the same as for DEFS, with the difference that regional events will be the event of interest while distant and local events and deaths will be regarded as competing events.

### 11.2.7 Time to Loco-regional Event (TTLRE)

This analysis aggregates the two previous endpoints, TTRE and TTLE. Due to the similarity in the prognostic value of these two endpoints it was deemed appropriate to group them in a composite endpoint, thus performing the analysis on a higher number of events and consequently with an increased power.

TTLRE is defined as the time from randomization to the first date of local or regional progression while on primary therapy or recurrence after surgery.

The method of analysis will be the same as for DEFS, with the difference that local and regional events will be the events of interest while distant events and deaths will be regarded as competing events.

### 11.2.8 Overall Survival (OS)

OS is defined as the time from randomization to the date of death. Patients alive at the time of analysis will be censored at their last contact date.

OS will be analyzed in the same manner as for EFS.

### 11.2.9 Handling of missing values

With regard to the objective response rate, the same rules as defined for the primary endpoint will be applied.

As far as time to event endpoints are concerned (i.e. EFS, DEFS, TTLE, TTRE, TTLRE, OS), the ITT analysis will include all randomized patients, without any exception. Censoring rules are defined in each relevant paragraph.

### 11.3 TIMING OF ANALYSIS

The primary analysis will take place as soon as all ITT patients have completed the surgery phase. This is expected to occur approximately 30 months (~2.5 years) after the randomization of the first patient, given that the enrollment period was completed in 22 months. The preparation of the study report (FSR) will follow the analysis of the primary endpoint: only the results of the analysis of pathological and clinical response rate will be reported as efficacy endpoints in the first release of the FSR.

The first analysis of time to event endpoints will take place approximately 5 years after the randomization of the first patient. At this early stage the analysis will be underpowered since most of the patients will not have experienced the event of interest and it has the only aim of early spread of possible relevant results. The results of this analysis will be reported as first addendum to the FSR.

In order to get more mature data, each randomized patient will be followed until the occurrence of the event up to 10 years after randomization. When the time frame is completed the final analysis of time to event endpoints will be performed and reported as a final addendum.
12. **EXTENT OF EXPOSURE**

12.1 **TREATMENT DURATION AND GENERAL INFORMATION**

Summary statistics will be provided for treatment duration and total number of cycles received. The analysis will be carried out broken down by the type of treatment: taxane therapy, combination chemotherapy overall and type of combination therapy (AC, EC, FEC).

Treatment duration will be calculated as:
\[ \text{Start date of last cycle} + \text{<per protocol cycle duration>} - \text{first treatment date} \], where per protocol cycle duration is 28 days for the taxane sequence and 21 days for the chemotherapy combination.

Number and percentage of patients by type of combination chemotherapy received (i.e. AC or EC or FEC) will be provided.

The median follow-up time jointly with the first and last quartile, will be quantified by the reverse Kaplan-Meier method, according to which death (censor flag=1) censors the true but unknown observation time of a subject and censoring is an endpoint (censor flag=0) [14].

12.2 **CUMULATIVE DOSE AND DOSE INTENSITY**

Summary statistics will be reported for cumulative dose, absolute dose intensity and relative dose intensity.

The **cumulative dose** will be expressed in mg/m² and calculated as follows:
both for taxane and combination chemotherapy the cumulative dose will be calculated as the sum of the total dose (mg) reported at each day of administration divided by the BSA (m²) reported in the eCRF at screening.

The **absolute dose intensity** (mg/m²/week) will be calculated as:
\[ \text{cumulative dose/treatment duration}*7 \].

The **intended dose intensity** (mg/m²/week) will be calculated as follows:
- **Paclitaxel**: \[ 90 \text{ (mg/m²)} * 3 \text{ (planned no. of administrations per cycle)} * 4 \text{ (planned no. of cycles)} / 16 \text{ (planned weeks of treatment)} \]
- **Abraxane**: \[ 125 \text{ (mg/m²)} * 3 \text{ (planned no. of administrations per cycle)} * 4 \text{ (planned no. of cycles)} / 16 \text{ (planned weeks of treatment)} \]
- **Combination individual drugs**: \[ \text{planned dose (mg/m²)} * 4 \text{ (planned no. of cycles)} / 12 \text{ (planned weeks of treatment)} \], where planned dose is: 600 for 5-Fluorouracil, 90 for Epirubicin, 60 for Doxorubicin and 600 for Cyclophosphamide.

The **relative dose intensity** (%) is the ratio between the absolute and the intended dose intensity multiplied by 100; it is an analogous to the concept of compliance, values near 100% will indicate an administration of treatment as per-protocol.

12.3 **DOSE MODIFICATIONS**

Number and percentages of patients by dose reductions and delays will be provided together with reasons for modifications (hematological AE, non-hematological AE, both hematological and non-hematological, other). Within each combination chemotherapy, frequency distributions of patients with dose reductions overall and by drug will be reported.
13. SAFETY

Patients will be assessed for adverse events by clinical examination, questioning for symptoms of toxicity, laboratory assessments, vital signs, ECG and LVEF.

Toxicities will be assessed throughout the study according the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Event (CTCAE) Version 4.0.

13.1 ADVERSE EVENTS

Adverse events (AEs) will be coded by MedDRA dictionary (with version available at database cut-off) and the Preferred Term will be used for reporting purposes.

The analysis will address the Treatment Emergent Adverse Events (TEAE) defined as all events with onset date posterior to the date of first treatment administration or started before the first study drug administration but worsening in severity during the treatment period. For each treatment arm, the incidence of TEAEs will be grouped by System Organ Class (SOC) and by Preferred Term. Each patient will be counted once for each SOC and/or for each preferred term, and in the analysis by CTCAE grade patients will be analyzed according to the worst grade reported throughout the whole treatment period for that specific SOC/Preferred Term. AE will be also described in terms of period of occurrence: whole reporting period, taxane period.

The same analysis will be performed for treatment related TEAE, i.e. for those events judged as with suspected relationship to the study drug by the investigator. Treatment relationship will investigated separately for taxane therapy and for combination chemotherapy as a whole, without making explicit reference to which drug of the combination the investigator addresses the relationship.

Subset of relevant events such as serious AEs, AEs with severity grade 3-5, AEs leading to discontinuation of study drug will be analyzed and reported separately.

Frequency tables of deaths and causes of death will be presented. The time from last dose of treatment to date of death will be reported in individual listings.

13.2 LABORATORY DATA

In this study it was decided to collect only the laboratory data that are assessed on the same days of treatment administrations. Should there be some toxicities issues leading to treatment delay until the toxicity is resolved, no safety data will be collected in the eCRF in the days in between the scheduled days of administration. Consequently, the information collected in the eCRF is partial since only the worst toxicity for patients well tolerating treatment will be recorded.

To avoid to underestimate the safety profile of the study drugs, the usual shift tables by CTCAE grade will not be produced and the evaluation of the severity of laboratory data will rely mainly on the investigator’s reporting of the corresponding adverse event in the AE form.

Laboratory toxicities will be instead analyzed by the incidence of abnormalities (low/high with respect to normal ranges), for patients with at least one assessment post-baseline and at risk (e.g. a patient ‘low’ at baseline will not make part of the analysis of the incidence of low abnormalities but will be counted instead for the analysis of high abnormalities). This approach describes the toxicities occurred during the study in a more general fashion and entails a less distorted interpretation of the toxicity profile. For instance for a specific parameter, any toxicity of grade 1 detected on a scheduled day of chemotherapy infusion would be always classified correctly as ‘abnormal’ even if a patient experienced an unreported toxicity worst than grade 1 across the study, while in the latter circumstance a grade 1 would wrongly represent the worst grade for the relevant lab. test were an analysis by grade performed.
13.3 ECG, LVEF AND VITAL SIGNS

The incidence of ECG abnormalities after treatment initiation will be provided.

LVEF will be first summarized in terms of variation over baseline with regard to the worst value observed after treatment start:

- increase or no change
- decrease by < 10 points from baseline
- decrease by 10-19 points from baseline (grade 2)
- decrease by ≥ 20 points from baseline (grade 3)

A second analysis will take into account both the absolute value and the same amount of variation from baseline according to the following categories:

- LVEF ≤ 50% and no decrease by ≥ 10 points from baseline
- LVEF ≤ 50% and decrease by 10-19 points from baseline
- LVEF ≤ 50% and decrease by > 20 points from baseline
- LVEF < 40% and no decrease by ≥ 10 points from baseline
- LVEF < 40% and decrease by 10-19 points from baseline
- LVEF < 40% and decrease by > 20 points from baseline

Finally, the LVEF value will be analyzed as continuous variable and displayed by box and whiskers plot at each time of measurement. The same graphical analysis will be performed for diastolic and systolic blood pressure.
14. **APPENDIX 1 – TABLES OF SECTION 14 OF THE FINAL STUDY REPORT (FSR)**

Overall content of Section 14 of the CSR (see guideline ICH topic E3):

14.1 Patient Disposition, Protocol Deviations, Demographic Data and Baseline Characteristics

14.1.1 Patient Disposition

14.1.2 Protocol Deviations

14.1.3 Demographic Data

14.1.4 Baseline Characteristics

14.2 Treatment Compliance and Efficacy Data

14.2.1 Treatment Compliance

14.2.2 Efficacy Data

14.3 Extent of Exposure and Safety Data

14.3.1 Displays of Adverse Events

14.3.2 Listings of Deaths, Other Serious and Significant adverse Events

14.3.3 Narratives of Deaths, Other Serious and Significant Events

14.3.4 Abnormal Laboratory Value Listings

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## 15. APPENDIX 2 – LISTING OF SECTION 16.2 OF THE FINAL STUDY REPORT (FSR)

Overall content of Appendix 16.2 of the CSR (see guideline ICH topic E3):

- 16.2.1 Patient Disposition and Discontinuation
- 16.2.2 Protocol Deviations
- 16.2.3 Patients Excluded from Any Analysis Population
- 16.2.4 Demographic Data and Medical History including Tumor History
- 16.2.5 Compliance and/or Drug Concentration Data (if available)
- 16.2.6 Individual Efficacy Response Data
- 16.2.7 Adverse Events Listings
- 16.2.8 Listing of Individual Laboratory Measurements by Patient
- 16.2.9 Other Safety Evaluations
- 16.2.10 Other Listings

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