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CLINICAL STUDY PROTOCOL

AVATAXHER

An open-label, randomized, multicenter, phase II, non comparative, exploratory study on neoadjuvant treatment with trastuzumab plus docetaxel plus bevacizumab according to Positon Emission Tomography (PET) value modification in patients with early stage HER2 positive breast cancer.

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PROTOCOL APPROVAL

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Head of Medical Affairs	Dr. Yannick Plétan
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SYNOPSIS

TITLE	An open-label, randomized, multicenter, phase II, non comparative, exploratory study on neoadjuvant treatment with trastuzumab plus docetaxel plus bevacizumab according to Positon Emission Tomography (PET) value modification in patients with early stage HER2 positive breast cancer.
SPONSOR	Roche SAS Phase II
INDICATION	Early stage HER2 positive breast cancer
OBJECTIVES	**Primary objective** To ***assess*** the complete pathological response rate~~s~~ (evaluation according to Chevallier's criteria, review by an independent Committee) in patients with a relative change in [18F]-FDG tumoral uptake < 70% and randomized in the arm with trastuzumab plus docetaxel plus bevacizumab. **Secondary objectives** • <u>In the populations of the two randomized arms</u> (Arms A and B, patients with Δ SUV < 70%) o To describe the complete pathological response rates evaluated according to local procedures (according to Chevallier's criteria) and review by an independent Committee (according to Sataloff's criteria: patients of Arms A and B ; according to Chevallier's criteria: Arm B); o To describe the ultrasound response rate, rate of conservative surgery, disease-free survival, distant disease-free interval, local relapse-free interval and overall survival; o To search for predictive factors of response to treatment on the basis of angiogenesis biomarkers (biology and imaging); • <u>In the population of non randomized patients</u> (Standard Arm, patients with Δ SUV ≥ 70%) o To describe the complete pathological response rates [(evaluation according to local procedures (according to Chevallier's criteria) and review by an independent Committee (according to Sataloff's and Chevallier's criteria)]; o To describe the ultrasound response rate, rate of conservative surgery, disease-free survival, distant disease-free interval, local relapse-free interval and overall survival; • <u>For all patients</u> (Arms A, B and Standard) o To assess the role of PET in early detection of pathological response; o To search for predictive factors of response to treatment [on the basis HER2/RH status, scintigraphy and imaging results (PET and mammary DCE-US), and pharmacokinetics, immunology, and pharmacogenetics (only for patients who agree to participate, specific consent)] results]; o To assess the safety according to CTC-AE v 4.0.

TRIAL DESIGN	Open-label, randomized, multicenter, non comparative, phase II, exploratory study After a selection period, 4 phases are planned: **Phase 1** (6 weeks: 2 cycles every 3 weeks)**: Neoadjuvant treatment and Positon Emission Tomographies (PET) before randomization** 1. PET 1 (within 7 days before Cycle No 1) 2. Cycle 1: docetaxel IV (100mg/m²) + trastuzumab IV (8mg/kg) 3. PET 2 (less than 3 days before Cycle No 2) 4. Cycle 2: docetaxel IV (100mg/m²) + trastuzumab IV (6 mg/kg) 5. Local results of the Δ SUV. **Phase 2 Randomization** (15 weeks: 5 cycles every 3 weeks)**:** **Neoadjuvant treatment according to the Δ SUV** ➢ If Δ SUV < 70%: randomization 2:1 (arms A and B, respectively) • Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6mg/kg) + bevacizumab (15mg/kg) (Arm A) • Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6mg/kg) (Arm B) • Cycle 7: trastuzumab IV (6mg/kg) (Arms A and B) ➢ If Δ SUV ≥ 70%: standard of care • Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6mg/kg) (Standard Arm) • Cycle 7: trastuzumab IV (6mg/kg) (Standard Arm) **Phase 3 : Surgery and Adjuvant treatment** (around 35 weeks) In all treatment Arms (Arms A, B and Standard): ➢ Surgery, after the Cycle 7 and between 4 and 6 weeks after the treatment perfusion of Cycle 6 ➢ Trastuzumab: 6 mg/kg, 11 cycles) ➢ Radiotherapy (during 4-6 weeks, according to sites standard practices) ➢ Hormonal therapy (mandatory if positive hormone receptors) **Phase 4 : Post-treatment follow-up** (around 50 months) Each patient will be followed until the end of the study, defined as 5 years after her inclusion (*inclusion defined as the start of neoadjuvant treatment*).
NUMBER OF SUBJECTS	72 subjects to be randomized 135 subjects to be included
TARGET POPULATION	Early stage HER2 positive breast cancer who is scheduled to receive neoadjuvant therapy with the objective of conservative surgery (T2 and T3)

INCLUSION CRITERIA	**General inclusion criteria** • Woman • Age ≥ 18 years • Patient must have signed a written informed consent form prior to any study specific screening procedures • Patients able to undergo a pre-treatment PET and a second course PET • Affiliated to the "Sécurité Sociale" or beneficiary to such a regimen **Disease Specific Inclusion Criteria** • Patient with invasive, T2 or T3 and histologically confirmed breast cancer, who is scheduled to receive neoadjuvant therapy with the objective of conservative surgery • Nx ou N0 or N1 • HER2 positive (needle core biopsy only) assessed by ICH [(HER2 +++) or HER2 ++ (and FISH or CISH + or SISH+)] or FISH + or CISH + or SISH +, on the basis of ASCO 2007 criteria, with centralized review by an independent committee at the end of the study • Known hormone receptors status • Performance status (ECOG Scale): 0, 1 or 2
EXCLUSION CRITERIA	**Cancer related Exclusion Criteria** • Partially or totally lobular carcinoma • Inflammatory breast cancer • Bifocal tumor, with the exception of tumors for which the bifocality be discovered on an MRI, the second tumor being located in the same quadrant as the original tumor and for which the indication of a possible breast-conservative surgery after neoadjuvant treatment would not changed • Metastases • Previous treatment with chemotherapy, radiation therapy or hormonal therapy for a breast tumor • Previous history of cancer (other than curatively treated basal and squamous cell carcinoma of the skin and/or in-situ carcinoma of the cervix) relapsing within the 5 years before study entry or in situ contralateral breast carcinoma. **Haematological, biochemical and organ function** • Absolute neutrophil count (ANC) <1.2 x 10^9/L • Platelet count <100 x 10^9/L • Haemoglobin < 9.00 g/dL • Impaired liver function: Serum (total) bilirubin: >1.5 x ULN (except if Gilbert hemolysis); AST or ALT: >2.5 x ULN • Inadequate kidney function: serum creatinine > 1.25 ULN or creatinine clearance < 50 mL/min according to the Cockroft and Gault formula • Urine dipstick for proteinuria ≥ 2+. Patients discovered to have ≥ 2+ proteinuria on dipstick urinalysis at baseline should undergo a 24 hour urine collection and must demonstrate ≤ 1 g of protein in 24 hours

	• Patients not receiving anticoagulant medication and having an International Normalized Ratio (INR) >1.5 or an activated Partial Thromboplastin Time (aPTT) or PTT >1.5 x ULN within 7 days prior to first study treatment ([1]). *Note: Patients receiving full dose oral or parenteral anticoagulants may be included in the study as long as anticoagulant dosing has been stable for at least two weeks prior to study entry and the appropriate coagulation monitoring tests are within local therapeutic limits ([1]).* **Other Study Drug Related Exclusion Criteria** • Uncontrolled hypertension (systolic >150 mmHg and/or diastolic >100 mmHg), with or without anti-hypertensive medication. Patients with high initial blood pressure are eligible if entry criteria are met after initiation or adjustment of antihypertensive medication. • History of thrombotic disorders within the last 6 months prior to enrolment (i.e. cerebrovascular accident, transient ischaemic attacks). • History of abdominal fistula, tracheo-oesophageal fistula or any grade 4 non-gastrointestinal fistula, gastrointestinal perforation or intra-abdominal abscess within 6 months of enrolment. • History or evidence of inherited bleeding diathesis or coagulopathy. • Non-healing wound, active peptic ulcer or bone fracture. • Major surgery (including open biopsy), significant traumatic injury within 28 days prior to enrollment or anticipation of the need for major surgery during study treatment. • Minor surgery, including insertion of an indwelling catheter, within 24 hours prior to the first bevacizumab infusion ([2]). • Current or recent use of any non-steroidal anti inflammatory agent (aspirin > 325 mg/day), or anti aggregation agents (dypiridamole, ticlopidine, clopidogrel > 75 mg/day), within 10 days before the first administration of bevacizumab ([2]). **General Exclusion Criteria** • Severe resting dyspnea due to complications or oxygen dependency • Clinically significant (i.e. active) cardiovascular disease, i.e. myocardial infarction within the last 6 months before inclusion, unstable angina, congestive heart failure NYHA Class ≥ II, serious cardiac arrhythmia requiring medication during the study which might interfere with regularity of the study treatment or not controlled by medication. • LVEF ≤ 50% by local definition using MUGA or echo cardiogram • Patient suffering from an uncontrolled diabetes (> 11 mmoles/L) ([3]) • Diabetic patient treated with oral antidiabetics or insulin with an underlying cardiopathy at ultrasound. • Evidence of ongoing or active infection (requiring IV antibiotics), any other disease, neurological or metabolic dysfunction, physical examination finding or laboratory finding giving reasonable suspicion

[1] These criteria have to be assessed before the randomization if Δ SUV < 70%
[2] These criteria have to be assessed before the randomization if Δ SUV < 70%
[3] According to cautions related to PET

	of a disease or condition that contraindicates the use of an investigational drug or increases the patient's risk of treatment-related complications • Pregnant or lactating women • Patients with reproductive potential not willing to use effective method of contraception during the treatment period and for 6 months after the last bevacizumab administration • Treatment with any other investigational agent, or participation in another clinical trial within 28 days prior to enrolment. • Vaccination by modified live vaccines, vaccination against yellow fever during study treatment period • Patients with known infection with HIV, HBV, HCV • Patients with a known allergy or sensitivity to monoclonal antibodies (bevacizumab, trastuzumab), to murine proteins, to hormonal therapies, to Chinese hamster ovary cell products or other study chemotherapies (docetaxel) or any of their excipients. • Patients assessed by the investigator to be unable or unwilling to comply with the requirements of the protocol
STUDY DURATION	30 months of recruitment + 5 years follow-up per patient after her inclusion *(inclusion defined as start of the neoadjuvant treatment)*
INVESTIGATIONAL MEDICINAL PRODUCT DOSE/ ROUTE/ REGIMEN	• **Bevacizumab *** (neoadjuvant treatment, **4 injections**, Arm A, Cycles 3 to 6) 15 mg/kg (IV infusion) every 3 weeks. 90 minutes IV infusion for the 1st administration ; *then if well tolerated,* 60 minutes IV infusion for the 2nd administration; *then if well tolerated* 30 minutes IV infusion for the following doses. *Surgery will be performed at least 4 weeks after the last infusion of neoadjuvant bevacizumab treatment.*
OTHER THERAPIES	• **Docetaxel** (neoadjuvant treatment, **6 injections**, all Arms) 100mg/m² (IV infusion) every 3 weeks. • **Trastuzumab** (neoadjuvant / adjuvant treatment, **18 injections**, all Arms) Neoadjuvant treatment (7 cycles): Loading dose: 8 mg/kg (IV infusion) Subsequent doses: 6 mg/kg, every 3 weeks (IV infusion). Adjuvant treatment (11 cycles): 6 mg/kg, every 3 weeks (IV infusion). • **Surgery:** Mammary surgery • **Radiotherapy** (adjuvant): duration 4-6 weeks and dose and frequency according to site's standard practice. • **Hormonal therapy** (adjuvant) **(mandatory if the patient is hormone receptors positive)** will be introduced after the end of the radiotherapy period (*drug, frequency, duration are left at the investigator's discretion*).

PRIMARY ENDPOINT & JUSTIFICATION OF SAMPLE SIZE	**Primary Endpoint:** Pathological complete response rates. pCR will be evaluated post-surgery (Chevallier's criteria) by an independent Committee (for all patients). The calculation of the sample size is based on a single stage phase II study using the Fleming design. A rate of complete response < 15% is considered as insufficient to continue the assement of combination. A rate of complete response > 35% is considered as proof of efficacy of the combination. Accepting a risk of α = 5% (type I error, one-sided) and β = 5% (type II error), 48 assasable patients are necessary in the docetaxel + trastuzumab + bevacizumab arm. As the docetaxel + trastuzumab arm is the randomized standard arm, no formal sample size was computed. 24 patients will be included in this arm to permit a 2:1 randomization ratio. In all, 72 patients (docetaxel + trastuzumab + bevacizumab: 48 patients; docetaxel + trastuzumab: 24 patients) will allow to evaluate the pCR rates in these two randomized arms. According to the hypothesis that 60% of patients will have a Δ SUV < 70% before Cycle 2 of neoadjuvant treatment (Berriolo-Riedinger et al. 2008), 48 further patients (with Δ SUV $\geq$ 70%) are necessary (these patients will continue docetaxel + trastuzumab neadjuvant treatment). Then, the total of assessable patients is 120. Furthermore, approximately 135 subjects will be enrolled in the study taking into account that 10% of the enrolled patients will drop out before randomization, to obtain 72 randomized patients.
STUDY ASSESSMENTS - EFFICACY	**Primary Endpoint (non comparative):** **Rate of pathological complete response** in surgical specimens of mammary tissue and lymph nodes, according to Chevallier's classification and reviewed by an independent Committee. Grade 1: Disappearance of All Tumors Either in the Breast or in the Nodes Grade 2: Persistence of Carcinoma In Situ in the Breast Only. No Nodal Invasion Grade 3: Presence of Invasive Carcinoma With Stromal Alteration Grade 4: Presence of Invasive Carcinoma Without Modification pCR is defined as the absence of invasive cancer cells in both the primary site and lymph nodes (grade 1 and 2).

Secondary Endpoints (non comparative):

Rate of pathological complete response in surgical specimens of mammary tissue and lymph nodes, according to Chevallier's classification (local procedures).

Rate of pathological complete response in surgical specimens of mammary tissue and lymph nodes, according to Sataloff's classification (centralized review).

Tumor (T)

TA:	**TB:**	**TC:**	**TD:**
Total or near total therapeutic effect	**Subjectively >50% therapeutic effect but less than total or near total**	**<50% therapeutic effect but effect evident**	**No response**

Node (N)

NA:	**NB:**	**NC:**	**ND:**
No nodal metastasis and evidence of therapeutic effect	**No nodal metastasis or therapeutic effect**	**Nodal metastasis present with evidence of therapeutic effect**	**Nodal metastasis present without evidence of therapeutic effect**

pCR is defined as tumor A (TA)/node A (NA) or TA/NB.

Ultrasound Response (UR): a complete response includes the disappearance of all measurable and assessable disease (based on RECIST criteria without confirmation), with no new lesion.

Rate of Conservative Surgery (RCS): proportion of patients who undergo conservative surgery after the neoadjuvant treatment.

Local Relapse-Free Interval (LRFI): time to local recurrence following first administration of neoadjuvant treatment, local recurrence in the ipsilateral or controlateral breast following lumpectomy.

Disease-Free Survival (DFS): time to local recurrence following first administration of neoadjuvant treatment, local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, distant recurrence, controlateral breast cancer, second primary cancer (other than squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, colon carcinoma in situ, or lobular carcinoma in situ of the breast) or death from any cause.

	Distant Disease-Free Interval (DDFI): time to distant recurrence following first administration of neoadjuvant treatment. **Overall survival (OS)**: time from first administration of study treatment to death from any cause. For patients alive at study end (premature discontinuation or final visit), censoring will be the last known date alive.
- SAFETY	At each cycle, clinical and laboratory AE will be reported according to the Common Terminology for Adverse Events (CTCAE V4.0). Cardiac safety according to NYHA classification.
- OTHER ASSESSMENTS (BIOLOGY, GENOMICS, IMAGING)	Predictive factors of efficacy and safety (translational research studies) : • Pharmacokinetics • Immunology • Pharmacogenetics • Angiogenesis biomarkers: biology and imaging

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GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
aPTT	activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
BP	Blood pressure
CBC	Complete Blood Count
CHF	Congestive heart failure
CI	Confidence interval
CISH	Chromogenic in situ hybridization
CR	Complete Response
CRF	Case Report Form[s]
DCE-US	Dynamic Contrast-Enhanced UltraSonography
DDFS	Distant Disease Free Survival
DFS	Disease Free Survival
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
ESF	eligibility screening form
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
GGT	Gamma-Glutamyl Transférase
H_0	Null hypothesis
H_1	Alternative hypothesis
HER-2	Human Epidermal Receptor-2
ICH	International Conference on Harmonization
IHC	Immunohistochemistry

GLOSSARY OF ABBREVIATIONS

INR	International Normalised Ratio
ITT	intent to treat
IV	Intravenous
LRFS	Local Relapse Free Survival
LVEF	Left Ventricular Ejection Fraction
MRI	Magnetic Resonance Image
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute-Common Toxicity Criteria for Adverse Events
NSABP	National Surgical Adjuvant Breast Project
NYHA	New York Heart Association
OS	Overall survival
pCR	Pathological Complete Response
PD	Pharmacodynamic
PET	Positon Emission Tomography
PFS	Progression free survival
PS	Performance Status
PK	Pharmacokinetic
PR	Pulse rate
PST	Post surgery therapy
PTT	Partial Thromboplastin Time
Q3W	Every 3 weeks
RCS	Rate of Conservative Surgery
RECIST	Response Evaluation Criteria in Solid Tumors
RFI	Recurrence Free Interval
SAE	Serious Adverse Event
SD	Stable Disease
SISH	Silver In Situ Hybridisation

GLOSSARY OF ABBREVIATIONS

SUV	Standard uptake Value
TAP	Thorax Abdomen Pelvis
TCA	Temps de Céphaline Activée
TNM	primary tumor/regional lymph nodes/distant metastasis
ULN	Upper Limit of Normal
UR	Ultrasound Response
US	UltraSonography
VEGF	Vascular Endothelial Growth Factor
β-HCG	β-Human Chorionic Gonadotropin

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND STUDY RATIONALE

1.1 Background

1.1.1 HER2-positive breast cancer

Breast cancer is the most common malignancy in women living in industrialized countries, and the most common cause of female cancer deaths (Harbeck 2007). In France, every year 42,000 new cases are reported and 12,000 women die of breast cancer (Ferlay et al. 2007; Hill et al. 2008). The prognosis of breast cancer depends upon its stage at diagnosis.

Patients whose cancer cells are positive for HER2/neu (about 20-30%, Slamon et al. 1987) have more aggressive disease, high risk of recurrence or metastasis, and poor prognosis (King et al. 1985; Slamon et al. 1987; van de Vijver et al. 1988; Gusterson et al. 1992; Hynes et al. 1994; Menard et al. 2008). Furthermore, HER2-positive breast cancer, when treated with primary surgery, demonstrates an early peak of local relapse or metastasis (Menard et al. 2002). A link has also been reported between HER2 over expression and post-surgery growth stimulation of breast carcinoma cells (Tagliabue et al. 2003).

Thus, stages of cancer as well as specific predictive makers, such as HER2 status, determine a specific therapeutic management.

1.1.2 Neoadjuvant treatment in HER2-positive breast cancer

1.1.2.1 Neoadjuvant treatment in breast cancer

Breast neoadjuvant treatment, i.e. preoperative systemic chemotherapy, is aiming at reduction of breast tumor volume and conservative surgery which is the most important hope for the patients who accept neoadjuvant chemotherapy. Conservative surgery and loco regional control can be expected if maximal reduction in tumor size is obtained by chemotherapy (Fisher et al. 1997; Fisher et al. 1998; Chen et al. 2004). Adjuvant and neoadjuvant approaches have the same long term results if all the treatments (chemotherapy, surgery, radiotherapy, hormonal therapy) are applied correctly (Mauri et al. 2005).

Pathological complete response (pCR), is a short-term surrogate marker correlated with long term outcome (Kurosumi 2004; Guarneri et al. 2006) and is an end point commonly used in neoadjuvant trials evaluating novel combinations. Pathologic complete response can be described according various classifications in particular the one of Chevallier (Chevallier et al. 1993) and the one of Sataloff (Sataloff et al. 1995). Despite the advances achieved with primary systemic therapy to date, novel treatment approaches are

still necessary to enhance disease control and increase the proportion of patients achieving a pCR. The use of targeted therapy could be a means to reach this goal.

1.1.2.2 Trastuzumab in neoadjuvant and adjuvant treatment of HER2 positive breast cancer

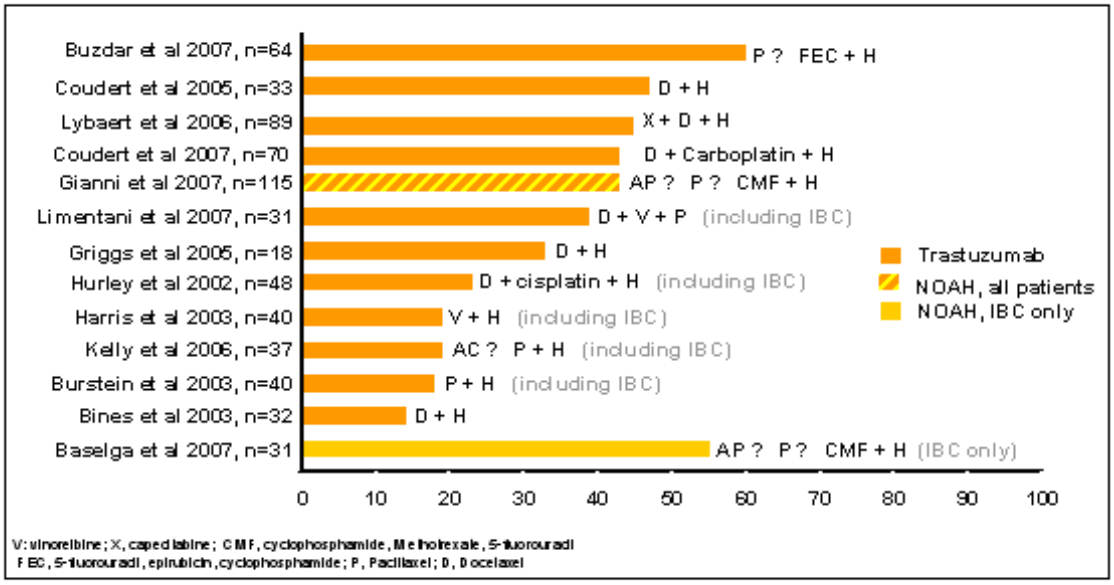
The first European approval of trastuzumab in breast cancer dates from March 2002.

The rationale for integrating trastuzumab into post-surgery treatment for HER2-positive breast cancer is based on the association between its use and the activation of immunologic functions (Clynes et al. 2000; Arnould et al. 2006), the inhibition of the HER2 extracellular domain (Sliwkowski et al. 1999) and the reduction of HER2 phosphorylation and downstream signaling (Molina et al. 2001).

Trastuzumab binds selectively to the HER2 receptor and provides significant clinical benefits (often including a survival advantage when combined with standard chemotherapy in HER2-positive breast cancer) both in the metastatic (Slamon et al. 2001; Marty et al. 2005; Gasparini et al. 2007) and adjuvant settings (Piccart-Gebhart et al. 2005; Romond et al. 2005). Furthermore, trastuzumab has a favorable safety and tolerability profile (Slamon et al. 2001; Marty et al. 2005; Piccart-Gebhart et al. 2005; Romond et al. 2005).

The addition of trastuzumab to 6 cycles of preoperative docetaxel (100 mg/m²) (Amat et al. 2003) has been shown to result in a pCR of 54% in patients with confirmed HER2-overexpressing (3+) and/or fluorescence in situ hybridization (FISH) positive tumors (Coudert et al. 2006). Briefly thirty-three patients were enrolled. The majority (79%) had T2 tumors, with 42% being N1/2. Twenty-nine patients completed six cycles of therapy. A complete or partial objective clinical response was seen in 96% (73% and 23%, respectively) of patients. Surgery was performed in thirty patients, breast conserving in 23 (77%) (Coudert et al. 2006). Tumor and nodal pCR was seen in 14 (47%) patients. Treatment was generally well tolerated. Grade 3/4 neutropenia occurred in 85% of patients. Only three patients withdrew prematurely due to toxicity. No symptomatic cardiac dysfunction was reported. These excellent results were confirmed in additional cohorts of patients (Ferrant et al. 2006; Favier et al. 2007). Furthermore, long term follow-up of patients receiving preoperative trastuzumab associated with either docetaxel or docetaxel + carboplatine, then adjuvant trastuzumab, shows disease-free survival (79%) and overall survival (92%) at 60 months (publication in progress). These results compare favorably with other long term follow-up of neoadjuvant trastuzumab studies (Buzdar et al. 2007; Gianni et al. 2008). Other phase II studies have studied preoperative docetaxel and trastuzumab (Van Pelt et al. 2003; Wenzel et al. 2004; Hurley et al. 2006; Sano et al. 2006; Coudert et al. 2007; Limentani et al. 2007). The dose of docetaxel (100 mg/m²) and the number of courses seem important to favor objective response, conservative surgery and pCR. Therefore 6 courses of docetaxel 100 combined with trastuzumab can be considered as one of the best standard neoadjuvant scheme in HER2 positive breast cancer because of immediate pCR results, conservative surgery rates and long term follow up results.

Figure 1: pCR brate in context: HER2-positive disease



1.1.2.3 Increasing the 50% pCR rate in HER2 positive breast cancer by adding bevacizumab

The first European approval of bevacizumab in breast cancer dates from March 2007.

In order to increase this 50% pCR rate result, with docetaxel and trastuzumab based chemotherapy, adding antiangiogenic compound could be considered. Angiogenesis is balanced between local pro-angiogenic and anti-angiogenic factors. VEGF is the most potent and specific known promoter of angiogenesis and is a key regulator of pathological angiogenesis such as that associated with tumor growth (Ferrara et al. 1997). Bevacizumab is a humanized monoclonal antibody to VEGF (rhuMAb VEGF) that blocks the binding of all isoforms of human VEGF to their receptors (Presta et al. 1997). Preclinical experiments indicate increased VEGF expression may in part mediate the biologically aggressive phenotype of HER2-overexpression.

In metastatic breast cancer, bevacizumab combined with chemotherapy (capecitabine or paclitaxel or docetaxel) is able to increase response rate (Miller 2003b; Miller et al. 2005; Miles et al. 2008) and progression-free survival (Miller 2003a; Miles et al. 2008). In the Avado study, the association of docetaxel 100 and bevacizumab 15 mg/kg/3w was the most efficient in response rate and progression-free survival (Miles et al. 2008). In inflammatory or locally advanced breast cancer, bevacizumab 15 mg/kg with doxorubicin and docetaxel was able to produce a 67% objective response rate (Wedam et al. 2006). In the neoadjuvant setting, bevacizumab would be able to improve complete pathological response, up to 41% pCR in the breast and 33% pCR in the breast and node as compared to 26% pCR, according to the NSABP 27 single arm, phase II study (Makhoul et al. 2008). Moreover, pre-clinical data and phase I-II data support that the combination of

bevacizumab and trastuzumab is synergistic and safe (Pegram et al. 2004; Pegram et al. 2006; Scheuer et al. 2006) when patients are chemotherapy naïve. Therefore adding bevacizumab to preoperative docetaxel trastuzumab could be a potential way to increase pCR and long term results of neoadjuvant treatment in HER2 positive breast cancer.

1.1.2.4 Relevance of an early Positon Emission Tomography for the neoadjuvant treatment of HER2 positive breast cancer patients.

In HER2 negative and HER2 positive tumors, the standard uptake value (SUV) decrease, studied with positon emission tomography (PET), is a strong predictor of pCR after only one course of chemotherapy (Berriolo-Riedinger et al. 2007). Other studies are confirmative of these data (Rousseau et al. 2006; Schwarz-Dose et al. 2008). In HER2 positive patients, the results are similar with different threshold values. Briefly, twenty four women with, non inflammatory, non metastatic, large or locally advanced breast cancer were studied. Two PET scans were performed at baseline before core biopsy and PST and just before the second course. The metabolic response of [18F]-FDG-PET was evaluated by the relative change in [18F]-FDG tumoral uptake (ΔSUV). To identify an optimal threshold for the prediction of the pathological response, receiver operating characteristics (ROC) analysis was performed for ΔSUV. After completion of chemotherapy, of the 24 breast tumours examined at surgery, 9 (38%) showed a pCR while 15 (62%) showed a non pCR. The relative decrease (ΔSUV) after the first course of neoadjuvant chemotherapy was significantly greater in the pCR group than in the non pCR group (-85 ± 19% versus –43 ± 25%; p = 0.001). An optimal cut-off value of –70% was found for ΔSUV. At this cut-off value, ΔSUV had a sensitivity of 89% while the specificity was 87%. From these results, a SUV decrease greater than 70% allows for the early identification of the highly responsive tumors, which will be completely eradicated by trastuzumab PST, with an accuracy of 88% (Berriolo-Riedinger et al. 2008; Berriolo publication in progress).

1.2 Study Rationale

Pathological complete response (pCR) is a short-term surrogate marker correlated with long term outcome and therefore it is a commonly used end point in neoadjuvant trials evaluating novel combination therapy.

The addition of trastuzumab to 6 cycles of preoperative docetaxel (100 mg/m²) has been shown to result in a pCR of 47%-54% of the patients with confirmed HER2-overexpressing.

Furthermore,

- Preclinical experiments indicate increased VEGF expression may in part mediate the biologically aggressive phenotype of HER2-overexpression;
- Pre-clinical data and phase I-II data support that the combination of bevacizumab and trastuzumab is synergistic and safe with chemotherapy-naïve patients.

It is the reason why adding bevacizumab to preoperative docetaxel + trastuzumab could result in a potential increase of pCR rate and long term benefit of neoadjuvant treatment in HER2 positive breast cancer.

The safety of bevacizumab has been well documented in clinical trials in HER2 positive breast cancer and its main side effects are well monitored by clinicians.

In this context, the main objective of this exploratory study is to assess the complete pathological response rate in the neo-adjuvant regimens docetaxel + trastuzumab + bevacizumab in patients with a high risk not to respond to chemotherapy. Then, this experimental regimen will be randomly assigned only to patients with a high risk of non-complete pathological response. The non-complete response after 6 courses of docetaxel + trastuzumab will be predicted by SUV decrease (threshold 70%) between initial and after one-course values. The response rates will be evaluated by Chevallier's criteria.

Alongside this main objective, this exploratory study will allow to search for predictive factors of treatment response (on the basis of pharmacokinetics, immunology, pharmacogenetics, and angiogenesis biomarkers).

2. OBJECTIVES

2.1 Primary Objectives

To assess the complete pathological response rates (evaluation according to Chevallier's criteria, review by an independent Committee) in patients with a relative change in [18F]-FDG tumoral uptake < 70% and randomized in the arm with trastuzumab plus docetaxel plus bevacizumab.

2.2 Secondary Objectives

- In the populations of the two randomized arms (Arms A and B, patients with Δ SUV < 70%)
    - To describe the complete pathological response rates evaluated according to local procedures (according to Chevallier's criteria) and review by an independent Committee (according to Sataloff's criteria: Arms A and B; Chevallier's criteria: Arm B);
    - To describe the ultrasound response rate, rate of conservative surgery, disease-free survival, distant disease-free survival, local relapse-free survival and overall survival;
    - To search for predictive factors of response to treatment on the basis of angiogenesis biomarkers (biology and imaging),
- In the population of non randomized patients (Standard Arm, patients with Δ SUV ≥ 70%)
    - To describe the complete pathological response rates [(evaluation according to local procedures (according to Chevallier's criteria) and review by an independent Committee (according to Sataloff's and Chevallier's criteria)];
    - To describe the ultrasound response rate, rate of conservative surgery, disease-free survival, distant disease-free survival, local relapse-free survival and overall survival;
- For all patients (Arms A, B and Standard)
    - To assess the role of PET in early detection of pathological response;
    - To search for predictive factors of response to treatment [on the basis of HER2/RH status, pathological complete response, imaging and scintigraphy results (PET and mammary DCE-US), pharmacokinetics, immunology, and pharmacogenetics (only for patients who agree to participate, specific consent) results];
    - To assess the safety according to CTC-AE v4.0.

3. STUDY DESIGN

3.1 Overview of Study Design

This is an open-label, multicentre, national phase II, and exploratory study assessing the efficacy and safety of docetaxel IV + trastuzumab IV with bevacizumab. According to relative change in [18F]-FDG tumoral uptake (ΔSUV) after the first neoadjuvant cycle, patients with ΔSUV < 70% will be randomized (2:1) in either docetaxel IV + trastuzumab IV + bevacizumab or docetaxel IV + trastuzumab IV whereas patients with ≥ 70% will receive standard care (docetaxel IV + trastuzumab IV).

Patient's selection will be hold between Day-28 and Day-1 (Day 1: first day of neoadjuvant treatment, cycle No 1). The selection will start by the signature of the informed consent by the patient.

After a selection period, 4 phases are planned (see Figure 2):

Phase 1: Neoadjuvant treatment and Positon Emission Tomographies (PET) before randomization (6 weeks: 2 cycles every 3 weeks)

1. PET 1 (within 7 days before Cycle No 1)
2. Cycle 1: docetaxel IV (100mg/m²) + trastuzumab IV (8mg/kg)
3. PET 2 (less than 3 days before Cycle No 2)
4. Cycle 2: docetaxel IV (100mg/m²) + trastuzumab IV (6 mg/kg)
5. Local results of the Δ SUV.

Phase 2: Randomization: Neoadjuvant treatment after randomization according to the Δ SUV (15 weeks: 5 cycles every 3 weeks)

- If Δ SUV < 70%: randomization 2:1 (arms A and B, respectively)
    - Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6mg/kg) + bevacizumab (15mg/kg) (Arm A)
    - Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6mg/kg) (Arm B)
    - Cycle 7: trastuzumab IV (6mg/kg) (Arms A and B)

- If Δ SUV ≥ 70%: standard of care
    - Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6mg/kg) (Standard Arm)
    - Cycle 7: trastuzumab IV (6mg/kg) (Standard Arm)

Phase 3: Surgery and Adjuvant treatment (around 32 weeks)

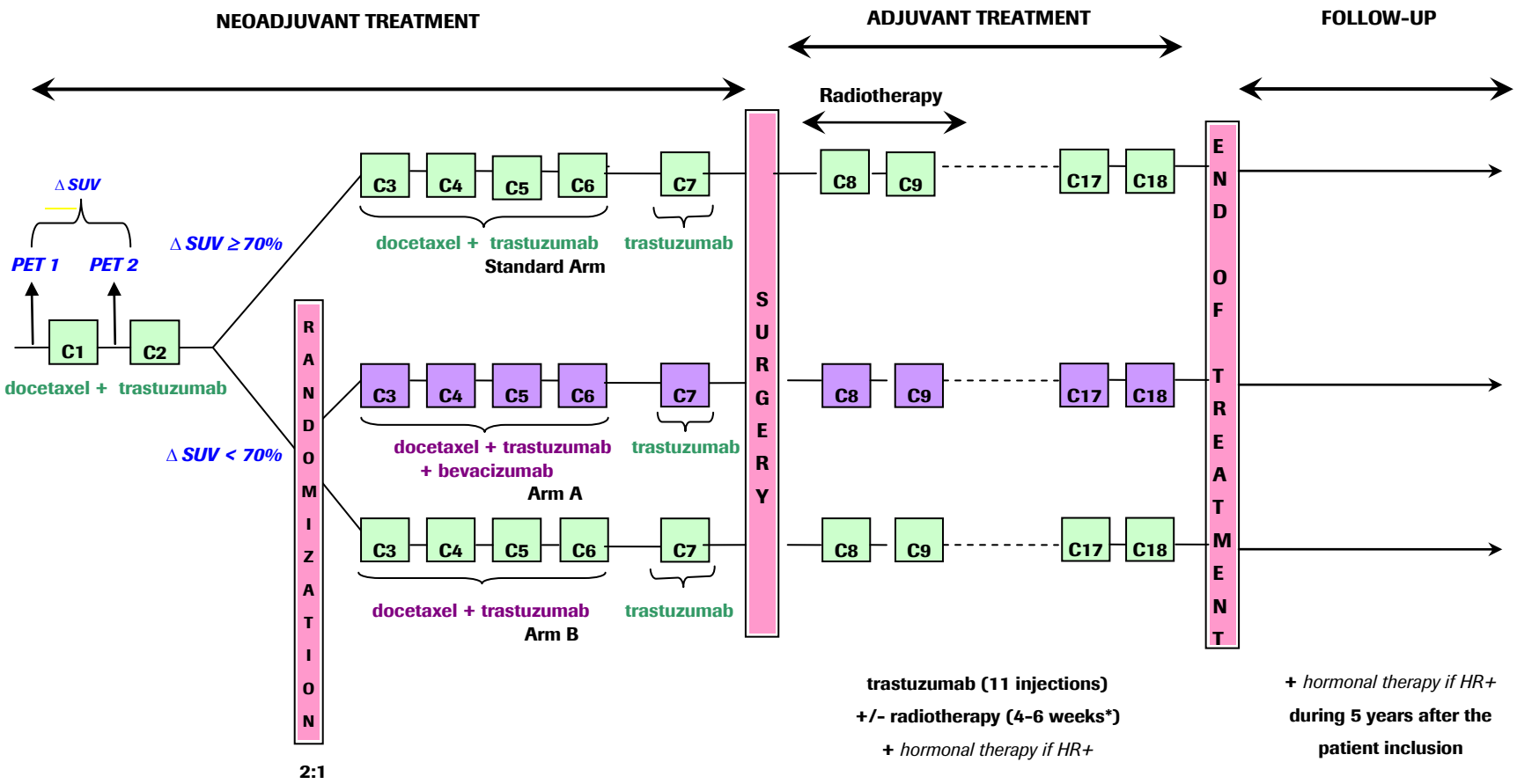
In all treatment Arms (Arms A, B and Standard):

- Surgery, after the Cycle 7 and between 4 and 6 weeks after the treatment perfusion of Cycle 6 (ie: 28 to 35 days after the last bevacizumab infusion for patients of Arm A) [Axillary procedure (sentinel node or axillary node dissection) and immediate breast reconstruction (in case of non conservative surgery) are allowed]
- Trastuzumab (6 mg/kg, 11 cycles)
- Radiotherapy (during 4-6 weeks, according to sites standard practices)
- with or without hormonal therapy, mandatory if positive hormone receptors

Phase 4: Post-treatment follow-up (around 50 months)

Each patient will be followed until the end of the study, defined as 5 years after her inclusion (inclusion defined as the start of neoadjuvant treatment).

Figure 2: Study Design



3.1.1 Rationale for Study Design

The design of this national, multicenter, open label, randomized, exploratory trial is a standard phase II study design assessing the neoadjuvant standard regimen of HER2+ breast cancer (docetaxel IV + trastuzumab IV) with the addition of bevacizumab.

This study will include patients with a HER2+ breast cancer who have no contraindication for the neoadjuvant standard treatment (docetaxel IV + trastuzumab IV), for bevacizumab IV (mainly, hemorrhagic and thrombolytic risks), and for the assessment technique supporting the randomization: [18F]-FDG-PET (uncontrolled diabetes).

As the standard uptake value (SUV) decrease of [18F]-FDG-PET is a predictor of the complete pathological response (pCR) after the first neoadjuvant cycle, it is of interest to assess the combination of bevacizumab with the standard therapy in patients with a potential high risk of non-complete pCR. Furthermore, the patients of high likelihood of complete pCR could be follow-up in this exploratory study as reference for the safety and the efficacy. Patients will be dispatched in potentially non- and complete responders according to their SUV decrease between the values before and after the first cycle of neoadjuvant treatment (cut-off: 70%).

Thus, only high-risk non-complete responders (patients with $\Delta SUV < 70\%$) will be randomized after the 2nd neoadjuvant cycle and they will receive four cycles of either standard regimen + bevacizumab (Arm A) or standard regimen alone (Arm B). The randomized patients without bevacizumab treatment (Arm B) will be followed-up in order to check that the calculation hypotheses for the bevacizumab Arm (Arm A) are not under or overestimated. The unbalanced randomization (2:1) will allow having a more important number of patients receiving the potentially most active therapeutic strategy. Patients with higher SUV decrease will continue the standard neoadjuvant regimen (Standard Arm).

Pathological response will be evaluated on operative specimens according to local procedures and centralized review, using Chevallier's criteria (primary endpoint) and according to centralized review, using Sataloff's criteria (secondary endpoint).

Thus, the advantage of this study design is that the relative change in [18F]-FDG tumoral uptake after the first neoadjuvant cycle will allow assessing the corrective action of early addition of bevacizumab to trastuzumab-docetaxel regimen, according to the predicted rate of pathological complete response. Furthermore, the efficacy and safety of early addition of bevacizumab to standard regimen will be assessed with other early response criteria (ultrasound response and rate of conservative surgery), and with the usual recurrence and survival criteria after five-year follow-up, in a large cohort of patients.

Alongside this main objective, this exploratory study will allow to search for predictive factors of treatment response, on the basis of HER2/RH status, pathological complete response, imaging and scintigraphy results (PET and mammary DCE-US), and

pharmacokinetics, immunology, and pharmacogenetics (only for patients who agree to participate, specific consent)] study results. Predictive factors of response to treatment will also be searched on angiogenesis biomarkers (biology and imaging, in one investigating center.

3.1.2 Rationale for Dose Selection

Based on Phase I and II dose regimens of bevacizumab, Wedam et al. [54] performed a study to evaluate parameters of angiogenesis in 21 previously untreated patients with inflammatory and locally advanced breast cancer treated with neoadjuvant and adjuvant bevacizumab. Patients received bevacizumab at Cycle 1 (15 mg/kg on Day 1) followed by six cycles of bevacizumab with doxorubicin (50 mg/m²) and docetaxel (75 mg/m²) every 3 weeks. This was the first clinical trial to demonstrate that bevacizumab has direct inhibitory effects on angiogenic parameters in tumor cells, on VEGF receptor activation, vascular permeability and flow, and induces apoptosis in tumor cells. Furthermore, in metastatic breast cancer, bevacizumab combined with chemotherapy (capecitabine or paclitaxel or docetaxel) is able to increase response rate or progression-free survival:

- In the Avado study, the association of docetaxel 100 mg/kg Q3w and bevacizumab 15 mg/kg/3w was the most efficient in terms of response rate and disease-free survival (Miles et al. 2008);

- In inflammatory or locally advanced breast cancer, bevacizumab 15 mg/kg with doxorubicin and docetaxel was able to produce a 67% objective response rate (Wedam et al. 2006).

So, for this study, we will assess bevacizumab at the dose of 15 mg/kg every 3 weeks (4 cycles: cycle 3 to 6 of neoadjuvant treatment). This is also the dose that was indicated in the marketing authorization of bevacizumab in metastatic breast cancer.

The dose of docetaxel used in this study (100 mg/m²) is within the range currently approved and recommended in the US, European Union and other countries (60-100 mg/m²) for the treatment of patients with breast cancer. The chosen dose of trastuzumab (8 mg/kg loading dose, followed by 6 mg/kg maintenance every three weeks) corresponds also to the usual schedule for this treatment.

3.1.3 End of Study

The end of study is **five years after patient's inclusion** (last visit of the last patient), the inclusion being defined as start of the neoadjuvant treatment.

3.2 Number of Subjects / Assignment to Treatment Groups

The patients with Δ SUV < 70% will be randomized (2:1) to received either docetaxel + trastuzumab + bevacizumab or docetaxel + trastuzumab.

In all, 72 patients (docetaxel + trastuzumab + bevacizumab: 48 patients; docetaxel + trastuzumab: 24 patients) will allow to describe the pCR rates in the two randomized arms.

According to the hypothesis that 60% of patients will have a Δ SUV < 70% before Cycle 2 of neoadjuvant treatment (Berriolo-Riedinger et al. 2008), 48 further patients (with Δ SUV ≥ 70%) are necessary (these patients will continue docetaxel + trastuzumab neadjuvant treatment). The total of assessable patients is 120.

Furthermore, approximately 135 subjects will be enrolled in the study taking into account that 10% of the enrolled patients will drop out before randomization (after the second PET), to obtain ***72*** randomized patients.

3.3 Centers

Approximately 30 French sites from public and private oncology centers will participate in this national trial.

All participating oncology centers will be specialized in breast cancer treatment and be able to participate in translational research studies. The centers must be able to process, temporarily store and ship specimens according to the specifications provided. Centers must have convenient patient access to radiotherapy, scintigraphy and imagery expertise and facilities, in particular DCE-US ("*dynamic contrast-enhance ultrasound*") and positon emission tomography (PET).

4. STUDY POPULATION

Under no circumstances are subjects who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.1 Overview

This study will include HER2 positive breast cancer female patients to be treated by trastuzumab + docetaxel combination neoadjuvant therapy. Among them, patients with early predicted non-complete pCR by [18F]-FDG-PET will be randomized in treatment groups with or without bevacizumab.

4.2 Inclusion Criteria

General inclusion criteria:

1. Woman
2. Age ≥ 18 years
3. Patient must have signed a written informed consent form prior to any study specific screening procedures
4. Patients able to undergo a pre-treatment PET and a second course PET
5. Affiliated to the "Sécurité Sociale" or beneficiary to such a regimen

Disease specific inclusion criteria:

1. Patient with invasive, T2 or T3 and histologically confirmed breast cancer, who is scheduled to receive neoadjuvant therapy with the objective of conservative surgery (see Appendix 2)
2. Nx ou N0 or N1 (see Appendix 2)
3. HER2 positive (needle core biopsy only) assessed by ICH [HER2 +++ or HER2 ++ (and FISH or CISH + or SISH+)] or FISH + or CISH + or SISH +, on the basis of ASCO 2007 criteria (see Appendix 3)
4. Known hormone receptors status.
5. Performance status (ECOG Scale, see Appendix 4): 0, 1 or 2

4.3 Exclusion Criteria

Cancer related Exclusion Criteria:

1. Partially or totally lobular carcinoma
2. Inflammatory breast cancer
3. Bifocal tumor, with the exception of tumors for which the bifocality be discovered on an MRI, the second tumor being located in the same quadrant as the original tumor

and for which the indication of a possible breast-conservative surgery after neoadjuvant treatment would not changed
4. Metastases
5. Previous treatment with chemotherapy, radiation therapy or hormonal therapy for a breast tumor
6. Previous history of cancer (other than curatively treated basal and squamous cell carcinoma of the skin and/or in-situ carcinoma of the cervix) relapsing within the 5 years before study entry or in situ contralateral breast carcinoma.

Haematological, biochemical and organ function:

1. Absolute neutrophil count (ANC) < 1.2 x 10^9/L
2. Platelet count <100 x 10^9/L
3. Haemoglobin < 9.00 g/dL
4. Impaired liver function: Serum (total) bilirubin: > 1.5 x ULN (except if Gilbert hemolysis); AST or ALT: > 2.5 x ULN
5. Inadequate kidney function: serum creatinine > 1.25 ULN or creatinine clearance < 50 mL/min according to the Cockroft and Gault formula
6. Urine dipstick for proteinuria ≥ 2+. Patients discovered to have ≥ 2+ proteinuria on dipstick urinalysis at baseline should undergo a 24 hour urine collection and must demonstrate ≤ 1 g of protein in 24 hours
7. Patients not receiving anticoagulant medication and having an International Normalized Ratio (INR) >1.5 or an activated Partial Thromboplastin Time (aPTT) or PTT >1.5 x ULN within 7 days prior to first study treatment ([4]).
   Note: Patients receiving full dose oral or parenteral anticoagulants may be included in the study as long as anticoagulant dosing has been stable for at least two weeks prior to study entry and the appropriate coagulation monitoring tests are within local therapeutic limits ([4]).

Other Study Drug Related Exclusion Criteria

1. Uncontrolled hypertension (systolic > 150 mmHg and/or diastolic >100 mmHg), with or without anti-hypertensive medication. Patients with high initial blood pressure are eligible if entry criteria are met after initiation or adjustment of antihypertensive medication.
2. History of thrombotic disorders within the last 6 months prior to enrolment (i.e. cerebrovascular accident, transient ischaemic attacks).
3. History of abdominal fistula, tracheo-oesophageal fistula or any grade 4 non-gastrointestinal fistula, gastrointestinal perforation or intra-abdominal abscess within 6 months of enrolment.
4. History or evidence of inherited bleeding diathesis or coagulopathy.
5. Non-healing wound, active peptic ulcer or bone fracture.
6. Major surgery (including open biopsy), significant traumatic injury within 28 days prior to enrollment or anticipation of the need for major surgery during study treatment.
7. Minor surgery, including insertion of an indwelling catheter, within 24 hours prior to the first bevacizumab infusion ([4]).

[4] These criteria have to be assessed before the randomization if Δ SUV < 70%

8. Current or recent use of any non-steroidal anti inflammatory agent (aspirin > 325 mg/day), or anti aggregation agents (dypiridamole, ticlopidine, clopidogrel > 75 mg/day), within 10 days before the first administration of bevacizumab ([4]).

General Exclusion Criteria

1. Severe resting dyspnea due to complications or oxygen dependency
2. Clinically significant (i.e. active) cardiovascular disease, i.e. myocardial infarction within the last 6 months before inclusion, unstable angina, congestive heart failure NYHA Class ≥ II (see Appendix 5), serious cardiac arrhythmia requiring medication during the study which might interfere with regularity of the study treatment or not controlled by medication.
3. LVEF ≤ 50% by local definition using MUGA or echo cardiogram
4. Patient suffering from an uncontrolled diabetes (> 11 mmoles/L) ([5])
5. Diabetic patient treated with oral antidiabetics or insulin with an underlying cardiopathy at ultrasound.
6. Evidence of ongoing or active infection (requiring IV antibiotics), any other disease, neurological or metabolic dysfunction, physical examination finding or laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or increases the patient's risk of treatment-related complications
7. Pregnant or lactating women
8. Patients with reproductive potential not willing to use effective method of contraception during the treatment period and for 6 months after the last bevacizumab administration.
9. Treatment with any other investigational agent, or participation in another clinical trial within 28 days prior to enrolment.
10. Vaccination by modified live vaccines, vaccination against yellow fever during study treatment period
11. Patients with known infection with HIV, HBV, HCV
12. Patients with a known allergy or sensitivity to monoclonal antibodies (bevacizumab, trastuzumab), to murine proteins, to hormonal therapies, to Chinese hamster ovary cell products or other study chemotherapies (docetaxel) or any of their excipients.
13. Patients assessed by the investigator to be unable or unwilling to comply with the requirements of the protocol

4.4 Concomitant Medication and Treatment

All concomitant medication must be recorded on the CRF. Additionally, any diagnostic, therapeutic, or surgical procedure performed during the study period should be recorded, including the date, indication, and description of the procedure(s) and any clinical findings.

[5] According to cautions related to PET

4.4.1 Prohibited Concomitant treatments

The following concomitant treatments are PROHIBITED in this study:

- Any anticancer treatment other than the study medication *(except for hormonal therapy if positive receptors)*; the use of other chemotherapeutic agents, biological agents or investigational drugs for breast cancer is forbidden during the study treatment and follow-up period.
- Other radiotherapy (except radiotherapy period planned in the protocol), as long as the patient has no recurrence.
- Vaccination by modified live vaccines, vaccination against yellow fever during study treatment period

Any anticancer treatment (including any radiotherapy) that is administered to patients withdrawn from the study treatment period for toxicity should be reported in the CRF to allow the follow-up of recurrence and survival.

4.4.2 Concomitant treatments to be avoided or requiring close monitoring

The following medications could interact with or exacerbate the side effects of bevacizumab, trastuzumab and/or docetaxel

- Drugs that could interact with bevacizumab

No formal drug interaction studies with other antineoplasic agents have been conducted. Existing data suggests that bevacizumab does not affect the pharmacokinetics of docetaxel or trastuzumab to a clinically relevant extent.

- Drugs that require close monitoring if they are administered with bevacizumab

To date, the most severe toxicities seen with bevacizumab have been hemorrhage, thrombosis and gastrointestinal perforation. For this reason, the use of anti-coagulant agents is limited.

- Patients with chronic, daily treatment with aspirin (> 325 mg/day) or non-steroidal anti-inflammatory agents that inhibit platelet function at the dose used (for treating chronic anti-inflammatory diseases) **at baseline cannot be included in the study** (*see inclusion and exclusion criteria*).
- **Prophylactic dose** of coumarin derivatives, heparin and low molecular weight heparin **is allowed at baseline and during study treatment**, as is low-dose aspirin (< 325 mg/day), occasional use of non-steroidal anti-inflammatory medication, or regular use of non-steroidal anti-inflammatory medication of the kind known not to inhibit platelet function.

  The INR of patients treated with prophylactic dose of anticoagulants must be regularly checked and the dose of anticoagulant administered should be adapted appropriately.

INR will be assessed at baseline before inclusion in the study for all patients.

- In patients who experience venous thrombolytic events at baseline and during study treatment (*see Table 6, section 6.1.3.3.*), **full dose anticoagulant treatment** (coumarin-derived anticoagulants or heparin, thrombolytic agents) **is allowed** and any information on this treatment (including doses) should be recorded in the CRF.

  INR must be checked at least every second day the first week of treatment, at least twice a week in the following treatment weeks until a stable therapeutic level of INR has been achieved and at least every third week when the weekly dose has been established and INR remains stable at this dose.

  - Drugs that could interact with docetaxel:

In vitro studies have shown that the metabolism of docetaxel may be modified by the concomitant administration of compounds that induce, inhibit, or are metabolized by cytochrome P450 3A4, such as cyclosporine, terfenadine, ketoconazole, erythromycin, and troleandomycin. Caution should be exercised with these drugs when treating patients receiving docetaxel as there is a potential for a significant interaction.

In vivo investigations show that caution should be exercised when administering ketoconazole to patients as concomitant therapy since there is a potential for a significant interaction.

More details can be found in the SPC of docetaxel.

- Drugs that could interact with trastuzumab:

Existing data suggests that trastuzumab does not affect the pharmacokinetics of docetaxel or bevacizumab to a clinically relevant extent.

4.4.3 Concomitant treatments allowed

Concomitant medications should be avoided except for **analgesics, chronic treatments for pre-existing medical conditions**, or agents required for life-threatening medical problems.

Any concomitant therapy (including **steroids** as **antiemetics** with docetaxel or premedication, **growth factors, proteins and procedures**, etc that is not prohibited in the protocol, is at the investigator's discretion and should be reported in the CRF.

Treatments with anthracyclins are not prohibited for patients who will not reach a complete histological response (after neoadjuvant treatment); this potential adjuvant treatment will be prescribed at the investigator's discretion and should be reported in the CRF.

Any new diagnosis, treatment or surgical intervention arising during the study treatment should be recorded in the CRF, including date, indication, description of the surgery and any other clinically pertinent information.

4.5 Criteria for Premature Withdrawal

Patients have the right to withdraw from the study treatment at any time for any reason. The Investigator also has the right to withdraw patients from the study treatment in the event of:

- Intercurrent illness
- Adverse events
- Pregnancy
- Treatment failure
- Patient non-compliance with study procedures
- Administrative reasons or other reasons.

An excessive rate of withdrawals can render the study un-interpretable. Therefore, unnecessary withdrawal of patients should be avoided

The Investigator should contact the patient either by telephone or through a personal visit or a responsible relative must be contacted to determine as completely as possible the reason for the withdrawal. A complete final evaluation of safety should be performed 28 days from the last dose of study treatment and a reason for the withdrawal should be obtained. If the reason for withdrawal is an adverse event or an abnormal laboratory test result, the principal specific event or test will be recorded on the eCRF.

4.6 Replacement Policy (Ensuring Adequate Numbers of Evaluable Subjects)

4.6.1 For Subjects

A patient that is prematurely withdrawn from the study period treatment, for whatever the reason, will not be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow recruitment.
- Poor protocol adherence.

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1: Schedule of Assessments: Screening and neoadjuvant treatment

	Screening		Inclusion	Neoadjuvant treatment						Preoperative assessment	
Calendar in weeks	W-4 to W-1	W-1 to D0	J1 / W1	W3	W4	W7	W10	W13	W16	W19	W20
Cycles			C1		C2	C3	C4	C5	C6	C7	
Informed consent	●										SURGERY [h]
Specific informed consent	●										
Demographics	●										
Medical history	●										
Physical examination	●	●	●		●	●	●	●	●	●	
Vital signs (temperature, BP), weight, PS		●	●		●	●	●	●	●	●	
Haematology and biochemistry [a]		●	●		●	●	●	●	●	●	
Urinalysis for proteinuria (dipstick) [b]		●	●		●	●	●	●	●	●	
Coagulation (INR, TCA) [c]		●	●		●	●	●	●	●	●	
Pregnancy test		●	<---------- *if clinically indicated* ---------->								
Blood samples for translational research studies			●		●	●	●	●	●		
Tumor assessment (HER2 status, hormone status)	●										
Tumor assessment (pCR)											●
LVEF (MUGA or echocardiogram) [d]	●							●			DAY 28 TO DAY 35 POST C6
ECG	●										
Positon Emission Tomography [e]		**PET 1**		**PET 2**							
Abdominal US+chest-X-ray (if abnormal + scanner TAP)	●	<---------- *if clinically indicated* ---------->								●	
Bone scintigraphy	●	<---------- *if clinically indicated* ---------->								●	
Bilateral mammary ultrasound	●	<---------- *if clinically indicated* ---------->								●	
Mamography	●										
Mammary DCE-US [f]		●				● [f]	● [f]				
Clinical assessment of tumor	●					●			●		
Inclusion/exclusion criteria	●	●				●					
Randomisation (before C3)						●					
Docetaxel [g]			●		●	●	●	●	●		
Trastuzumab [h]			●		●	●	●	●	●	●	
Bevacizumab (arm **A only**)[i]						●	●	●	●		
Concomitant medications	<---------- uninterrupted ---------->										
AEs and all SAEs	<---------- must be collected up to 28 days after last treatment administration ---------->										
AEs of special interest (Arm A only) [j]		<------- must be collected up to 6 months after le last injection of bevacizumab ------->									

[a] : CBC, platelets, haemoglobin *and* glycemia, serum creatinine, creatinine clearance, AST, ALT, alkaline phosphatase, GGT, serum bilirubin, LDH

[b] : Dipstick + 24 h hour urine proteinuria if necessary for screening phase and reassess if positive at previous evaluation

[c] : For screening phase and reassess only for patients receiving a coumarin anticoagulant treatment

[d] : Every 3 months during trastuzumab treatment and 6 months, 12 months and 24 months after the end of trastuzumab treatment.
The same method should be used throughout the whole study duration

[e] : **TEP 1** within 7 days before C1 and at least 10 days after the biopsy and **TEP 2** less than 3 days before C2. On the basis of TEP results, the patient will withdraw from the study if the uptake of [18F]-FDG is low (SUV ≤ SUV of controlateral mammary parenchyma) or if a metastasis in mediastinal lymph node(s) is detected; the patient will remain in the study if a metastasis in ipsilateral internal mammary or supraclavicular lymph node(s) is detected.

[f] : A mammary DCE-US will be performed at screening within the week before inclusion for all patients in equiped centres. The following mammaries DCE-US (at C3 and C4) will be only performed for patients of Arms A and B and only if contrast enhancement of the 1st mammary at screening is > 50%. These following mammaries DCE-US must be performed within 7 days before C3 and within 2 days before C4

[g] : 6 cycles (every 3 weeks) docetaxel IV (100 mg/m^2) C1 to C6 for all patients

[h] : 18 cycles (every 3 weeks) trastuzumab IV (8mg/kg, loading dose) at C1 and trastuzumab IV (6 mg/kg) from C2 to C18 for all patients
→ 7 cycles neoadjuvant then 11 cycles adjuvant = 18 infusions in total

[i] : 4 cycles (every 3 weeks) bevacizumab IV (15 mg/kg) from C3 to C6 after randomization for patients with Δ SUV < 70 %

[j] : neutropenia, wound healing complications, proteinuria, bleeding/haemorrhage, hypertension, thromboembolic events (venous or arterial), congestive heart failure, gastrointestinal perforation, fistulae, Reversible Posterior Leucoencephalopathy Syndrome (RPLS)

[h] : Axillary procedure (sentinel node or axillary node dissection) and immediate breast reconstruction (in case of non conservative surgery) will be performed according to sites standard practices

Table 2: Schedule of Assessments: Surgery – Adjuvant treatment (including Radiotherapy)

	post operative assessment		Post * radiotherapy assessment	Adjuvant treatment								End of treatment or withdrawal	Post treatment follow-up
calendar	W22	W25	W28	W31	W34	W37	W40	W43	W46	W49	W52	W56	Every 6 months until 5 years
Cycles	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18		
Physical examination									●				
Vital signs (temperature, BP), weight, PS	●	●	●	●	●	●	●	●	●	●	●		
Haematology and biochemistry[a]			●						●			●	
Coagulation (INR, TCA) [b]	●	●	●	●	●	●	●	●	●	●	●	●	
Urinalysis for proteinuria (dipstick)[c]	●	●	●	●	●	●	●	●	●	●	●	●	
Pregnancy test	<---------- if clinically indicated ---------->												
LVEF (MUGA or echocardiogram) [d]	●				●				●			●	●(d)
Abdominal US+chest-X-ray (if abnormal + scanner TAP)	<---------- if clinically indicated ---------->								●	<-- if clinically indicated -->			●
Bone scintigraphy	<---------- if clinically indicated ---------->												if clinically indicated
Bilateral mammary ultrasound	<---------- if clinically indicated ---------->											●	● (Once a year)
Mamography	<---------- if clinically indicated ---------->											●	● (Once a year)
Clinical assessment of tumor recurrence									●			●	●
Trastuzumab [e]	●	●	●	●	●	●	●	●	●	●	●		
Radiotherapy [f]		<---------->											
+/- hormonal therapy				<----- mandatory if positive hormone receptors - according to site standard practices ----->									
Concomitant medications	<---------- uninterrupted ---------->												
Treatment for relapse only													uninterrupted
Aes and all SAE	<---------- must be collected up to 28 days after last treatment administration ---------->												
SAEs related to study													uninterrupted
Survival status	<---------- uninterrupted---------->												

[a] : CBC, platelets, haemoglobin *and* glycemia, serum creatinine, creatinine clearance, AST, ALT, alkaline phosphatase, GGT, serum bilirubin, LDH

[b] : Only for patients receiving a coumarin anticoagulant treatment

[c] : Only if abnormal during bevacizumab neoadjuvant treatment

[d] : At cycle 8 or between cycle 8 and 9 then every 3 months during trastuzumab treatment and 6 months, 12 months and 24 months after the end of trastuzumab treatment. The same method should be used throughout the whole study duration

[e] : All the patients receive 11 cycles of trastuzumab IV as adjuvant treatment, from C8 to C18 (6 mg/kg).
[f] : If performed : 4 to 8 weeks after surgery during 4 to 6 weeks according to site standard practices
* : This assessment must be practiced at the end of radiotherapy treatment according to site standard practices

5.1 Screening Examination and Eligibility Screening Form

All subjects must provide written informed consent before any study specific assessments or procedures are performed.

A specific informed written consent has to be obtained for translational studies.

Patient fulfilling all **inclusion and exclusion criteria** will be eligible for the study.

The screening examination should be performed between 28 and 1 day before Day1 (1st administration of study treatment), however some examination should be performed within 7 days (Table 1). Patients who fulfill all the inclusion and none of the exclusion criteria will be accepted into the study.

The following evaluations will be performed **within 28 days** prior to the day before D1:

- Demographics,
- Medical and surgical history, and history of previous cancer,
- Concomitant medications and diseases,
- Physical examination
- History of breast cancer, TNM clinical staging (see Appendix 2), tumor assessement (needle core biopsy only), HER 2 and hormone receptors status,
- ECG
- Validation of inclusion/exclusion criteria

Imaging:

- LVEF (MUGA or echocardiogram, the same method should be used throughout the whole study duration)
- Bilateral mammary ultrasound
- Mammography
- Extension assessment : - abdominal US +chest –X-ray (If abnormal + scanner TAP (Thorax Abdomen Pelvis)
  - bone scintigraphy.

HER 2 status will be assessed before inclusion by the local pathologist of each center and reviewed by a centralized reviewer after the last inclusion (*see section 10.3*). Centralized review will be assessed on tumor block prepared by the local pathologist during screening period (paraffin block).

Positon Emission Tomography (PET-1): within 7 days before C1 and at least 10 days after the biopsy (see Appendix 6)

The following evaluations will be performed **within 7 days** prior to the 1st administration of the neoadjuvant treatment:

- Vital signs (temperature, blood pressure [BP]), weight, performance status (PS) (see Appendix 4), physical examination

- Coagulation (INR, TCA) <u>for all patients</u>
- Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH*
- Urinalysis for proteinuria (dispstick + 24 h urine proteinuria if necessary, see Appendix 7)
- Blood pregnancy test (for all women of childbearing potential or amenorrheic for the last 24 months)
- Validation of inclusion/exclusion criteria

<u>Imaging:</u>

- Mammary DCE-US (equiped centres).

An Eligibility Screening Form (ESF) documenting the investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 Procedures for Enrollment of Eligible Subjects

Once a subject has fulfilled the entry criteria, she will at first entered in the neoadjuvant treatment period (2 first cycles of docetaxel and trastuzumab, *see section 5.3.1*) <u>prior</u> to randomization. As randomization is based upon the early response to the first chemotherapy cycle, the second PET (PET-2) to establish the local Δ SUV will be performed within 3 days before Cycle 2 (Table 1, see Appendix 6), allowing the **randomization prior to Cycle 3.**

For all subjects and on the basis of the PET results:

- The patient will withdraw from the study if the uptake of [18F]-FDG is low (SUV ≤ SUV of controlateral mammary parenchyma) or if a metastasis in mediastinal lymph node(s) is detected;

- The patient will remain in the study if a metastasis in ipsilateral internal mammary or supraclavicular lymph node(s) is detected.

For subjects with Δ SUV < 70% and before the randomization, the following criteria will be assessed:

- If minor surgery, including insertion of an indwelling catheter, within 24 hours prior to the first infusion, the infusion must be delayed for 24 hours;
- If current or recent use of any non-steroidal anti inflammatory agent (aspirin > 325 mg/day), or anti aggregation agents (dypiridamole, ticlopidine, clopidogrel > 75 mg/day), the infusion must be delayed for 10 days;
- If current or recent use of thrombolytic agent for therapeutic purposes, the infusion must be delayed for 10 days.

- If use of full dose oral or parenteral anticoagulants, the infusion must be delayed until a stable anticoagulant dosing for at least two weeks and appropriate coagulation monitoring tests within local therapeutic limits.

The only eligible subjects with Δ SUV < 70% will be randomly assigned to treatment groups.

These eligible patients will be randomized prior to Cycle 3 to one treatment group using a centralized, adaptative minimization method. The randomization will be unbalanced 2:1 (2 patients in arm A for 1 patient in arm B). Minization variables will be the centre, the primary tumour (T) from the TNM staging system, the hormone receptors status and the SBR grade.

Randomization will be performed via the e-CRF: www.avataxher-roche.org.

After validation of randomization criteria, patients will be randomized according to Δ SUV 70% cut-off:

- **If Δ SUV < 70%:** patients will be randomized to one neoadjuvant treatment group. The randomization will be unbalanced (2:1).
  - Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6 mg/kg) + bevacizumab (15 mg/kg) (Arm A)
  - Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6 mg/kg) (Arm B)
  - Cycle 7: trastuzumab IV (6 mg/kg) (Arms A and B)

- **If Δ SUV ≥ 70%:** patient will continue the standard-of-care neoadjuvant treatment:
  - Cycles 3 to 6: docetaxel IV (100mg/m²) + trastuzumab IV (6 mg/kg) (Standard Arm)
  - Cycle 7: trastuzumab IV (6 mg/kg) (Standard Arm)

5.3 Clinical Assessments and Procedures

5.3.1 Neoadjuvant treatment Period - Before randomization (2 cycles)

- **The 2nd Positon Emission Tomography will be performed within 3 days before Cycle 2** (see Appendix 6)

  The Δ SUV (between the 2 PET) will be assessed in each centre before the patient's randomization (Δ SUV 70% cut-off).

- **Before each cycle,** following evaluations will be performed (Table 1):
    - Vital signs (Temperature, BP), weight, PS (see Appendix 4), physical examination,
    - Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH*
    - Urinalysis for proteinuria (*if positive at previous evaluation*, see Appendix 7)
    - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*).
    - Blood pregnancy test (*if clinically indicated*)
    - Adverse Events
    - Other concomitant medications.

Supplementary blood samples will be taken **at inclusion prior to the 1st administration of the neoadjuvant treatment**, only for patients who agreed to participate to translational research studies: pharmacokinetics, immunology, pharmacogenetics (*specific informed consent form needeed*), angiogenesis studies (see Appendix 11).

- **Imaging**
    - Bilateral mammary ultrasound will be performed at any time *if clinically indicated.*
    - Extension assessments [abdominal US + chest-X-ray (+ scanner TAP if abnormal) and bone scintigraphy] will be also performed at any time *if clinically indicated.*

  Imaging can be performed on the same day of the study drugs administration but before the administration.

5.3.2 Neoadjuvant treatment period - After randomization according to the Δ SUV (5 cycles)

- **Before each cycle (*from Cycle 1 to Cycle 6),*** following evaluations will be performed (Table 1):
    - Vital signs (temperature, BP), weight, PS (see Appendix 4), physical examination,
    - Clinical assessment of tumor response (at Cycle 3 and 6 only)
    - Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH*
    - Urinalysis for proteinuria (*if positive at previous evaluation,* see Appendix 7)

- Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*).
- Blood pregnancy test (*if clinically indicated*)
- Adverse Events
- Other concomitant medications.

Supplementary blood samples will be taken only for patients who agreed to participate to the following translational research studies: pharmacokinetics and angiogenesis studies (C3 for all and C4 for Arm A) (see Appendix 11).

- During the **Preoperative assessment visit (W19) and before Cycle 7 (the last Cycle of systemic neoadjuvant treatment with trastuzumab), the following evaluations will be performed (Table 1):**
  - Vital signs (temperature, BP), weight, PS (see Appendix 4), physical examination,
  - Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH*
  - Urinalysis for proteinuria (*if positive at previous evaluation,* see Appendix 7)
  - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*)
  - Blood pregnancy test (*if clinically indicated*)
  - Adverse Events
  - Other concomitant medications
  - Imaging:
    - Bilateral mammary ultrasound
    - Extension assessments [abdominal US + chest-X-ray (+ scanner TAP if abnormal) and bone scintigraphy].

- **Imaging**
  - **LVEF** will be performed before Cycle 5 only. LVEF assessments will be performed every 3 months during trastuzumab treatment and 6, 12, and 24 months after the end of trastuzumab treatment (the same method as at screening visit)
  - **Mammary ultrasound** will be performed at any time *if clinically indicated.*
  - **Mammary DCE-US** (at Cycles 3 and 4) will be only performed for patients of Arms A and B and only if contrast enhancement of the baseline mammary is > 50%. These following mammaries DCE-US must be performed within 7 days before C3 and within 2 days before C4

- **Extension assessments** [abdominal US + chest-X-ray (+ scanner TAP if abnormal) and bone scintigraphy] will be performed at any time *if clinically indicated*

Imaging can be performed on the same day of the study drugs administration but before the administration.

5.3.3 Surgery and Adjuvant treatment Period

Adjuvant therapy cycles will go on every 3 weeks until Cycle 18.

SURGERY

Surgery will be performed after the Cycle 7 for all patients and at least 4 weeks after the last infusion of neoadjuvant bevacizumab treatment (Arm A, C6).

- **Surgery** will be performed at W20 (between D28 and D35 after the Cycle 6). The type of breast surgery will be established by the surgeon and at the centre's discretion: axillary procedure (sentinel node or axillary node dissection) and immediate breast reconstruction (in case of non conservative surgery). The pathological response in operative specimens of mammary tissue and lymph nodes will be evaluated according to Chevallier's ***and Sataloff's*** classification (see Appendix 8).

- **Post operative assessments** will be performed according to Table 2, at Cycle 8 (1st Cycle of systemic adjuvant treatment with trastuzumab):
    - Vital signs (temperature, BP), weight, PS (see Appendix 4),
    - Urinalysis for proteinuria (dipsticks, *only in Arm A and if abnormal during the bevacizumab neoadjuvant treatment* (see Appendix 7)
    - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*)
    - Blood pregnancy test (*if clinically indicated*)
    - Imaging:
        - LVEF (MUGA or echocardiogram (the same method should be used throughout the whole study duration) at Cycle 8 or between Cycles 8 and 9,
        - Extension assessments [abdominal US + chest-X-ray (+ scanner TAP if abnormal), bone scintigraphy, bilateral mammary ultrasound and mammography], will be performed at any time *if clinically indicated*. Imaging can be performed on the same day of the study drugs administration but before the administration.

ADJUVANT TREATMENT

Radiotherapy

If indicated, patients will undergo radiotherapy, 4 to 8 weeks after surgery. Radiotherapy will last for 4 to 6 weeks, according to site standard practices.

During the radiotherapy period, the following evaluations will be performed (Table 2):

- **Before Cycle 9**
  - Vital signs (temperature, BP), weight, PS (see Appendix 4),
  - Urinalysis for proteinuria (dipsticks, *only in Arm A and if abnormal during the bevacizumab neoadjuvant treatment* (see Appendix 7)
  - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*)
  - Blood pregnancy test (*if clinically indicated*)
  - Adverse Events
  - Other concomitant medications
  - Imaging:
    - Extension assessments [abdominal US + chest-X-ray (+ scanner TAP if abnormal), bone scintigraphy, bilateral mammary ultrasound and mammography], will be performed at any time *if clinically indicated.* Imaging can be performed on the same day of trastuzumab administration but before the administration.

- **Post radiotherapy assessment at Cycle 10** (at the end of radiotherapy, according to site standard practices):
  - Vital signs (temperature, BP), weight, PS (see Appendix 4),
  - Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH*
  - Urinalysis for proteinuria (dipsticks, *only in Arm A and if abnormal during the bevacizumab neoadjuvant treatment* (see Appendix 7)
  - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*)
  - Blood pregnancy test (*if clinically indicated*)
  - Adverse Events
  - Other concomitant medications
  - Imaging:
    - Extension assessments [abdominal US + chest-X-ray (+ scanner TAP if abnormal), bone scintigraphy, bilateral mammary ultrasound and mammography], will be performed at any time *if clinically indicated.* Imaging can be performed on the same day of trastuzumab administration but before the administration.

Post-radiotherapy treatment

- **Before each cycle (Cycle 11 to Cycle 18),** following evaluations will be performed (Table 2):
  - Vital signs (temperature, BP), weight, PS (see Appendix 4),
  - Urinalysis for proteinuria (dipsticks, *only in Arm A and if abnormal during the bevacizumab neoadjuvant treatment* (see Appendix 7)
  - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*)
  - Blood pregnancy test (*if clinically indicated*)
  - Adverse Events
  - Other concomitant medications
  - Extension assessments
    - abdominal US + chest-X-ray (+ scanner TAP if abnormal). These assessments will also be performed at any time *if clinically indicated.*
    - bone scintigraphy, bilateral mammary ultrasound and mammography will be performed at any time *if clinically indicated.*
    - Imaging can be performed on the same day of the trastuzumab administration but before the administration.

- **Only before Cycles 12 and 16: LVEF** (MUGA or echocardiogram; the same method should be used throughout the whole study duration).

- **Only before Cycle 16:**
  - Physical examination,
  - Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH,*
  - Clinical assessment of tumor recurrence
  - Extension assessments
    - abdominal US + chest-X-ray (+ scanner TAP if abnormal).
    - imaging can be performed on the same day of the trastuzumab administration but before the administration.

- **28 days after the last infusion of trastuzumab (and if premature withdrawal)**, the following evaluations will be performed (Table 2):
    - Clinical assessment of tumor recurrence
    - Hematology and biochemistry: *CBC, platelets, haemoglobin, glycaemia, serum creatinine, creatinine clearance, alkaline phosphatase, GGT, serum bilirubin, AST, ALT, LDH,*
    - Urinalysis for proteinuria (dipsticks, *only in Arm A and if abnormal during the bevacizumab neoadjuvant treatment* (see Appendix 7)
    - Coagulation (INR and TCA) (*only for patients receiving a coumarin anticoagulant*)
    - Blood pregnancy test (*if clinically indicated)*
    - Adverse Events
    - Other concomitant medications
    - Imaging:
        - LVEF (MUGA or echocardiogram, the same method should be used throughout the whole study duration)
        - Mammography and bilateral mammary ultrasound
        - Extension assessments [abdominal US + chest-X-ray (+ scanner TAP if abnormal) and bone scintigraphy will be performed at any time *if clinically indicated.*

5.3.4 Post-Study Follow-Up

At the time of premature withdrawal or discontinuation due to disease progression or recurrence, treatment-related toxicity or other reasons, the patient will enter the **post-treatment follow-up period only if she has received at least one infusion** (Table 2). Patients will undergo the following evaluations every 6 months until 5 years after patient's inclusion:

- Clinical assessment of tumor recurrence
- Survival status
- Adverse Events (SAEs related to study drug and AEs of special interest),
- Imaging:
    - LVEF at 6, 12 and 24 months after last dose of trastuzumab (the same method should be used throughout the whole study duration)
    - Mammography and mammary ultrasound (once a year)
    - Extension assessments
        - abdominal US + chest-X-ray (+ scanner TAP if abnormal)
        - bone scintigraphy at any time *if clinically indicated.*

The patient may also undergo their usual visits at the centre without any specific reporting exept AEs. The treatment of possible recurrences will be performed according to site standard practices.

5.3.5 Tumor Response Criteria

5.3.5.1 Pathological response

The pathological response in operative specimens of mammary tissue and lymph nodes will be evaluated according to Chevallier's and Sataloff's classification (see Appendix 8) and reviewed by an independent Committee (*see Section 10.2*).

The tumor specimens will be to process, temporarily store and ship specimens according to the specifications provided (see Appendix 8).

5.3.5.2 Imaging response

Positon Emission Tomography (PET)

For all patients, the first PET will be realized within 7 days before the first administration of neoadjuvant treatment and the second less than 3 days before Cycle 2.

The PET procedures are detailed in Appendix 6.

The Δ SUV (between the 2 PET) will be assessed in each centre before the patient's randomization (Δ SUV 70% cut-off).

Mammary DCE-US

Tumor response will be evaluated during the neoadjuvant period.

In this study, tumor response will be measured by mammary DCE-US (equipped centres). For each subject, the same method of assessment and the same technique **must** be used to evaluate each lesion throughout the entire neoadjuvant treatment period.

The use of oral and IV contrast etc. should, as long as it is clinically possible, be kept consistent. Tumor measurements should be made by the same investigator/radiologist for each patient during the study to the extent that this is feasible.

Scheduling of tumor imaging assessments

Baseline total tumor burden must be assessed within the week before first dose of study drug treatment for all patients. Post-baseline assessments are to be performed at **Cycles 3 and 4 but only for patients of Arms A and B and only if contrast enhancement of the baseline mammary is > 50%. These following mammaries DCE-US must be performed within 7 days before C3 and within 2 days before C4**.

If there is suspicion of disease progression based on clinical or laboratory findings before the next scheduled assessment, an unscheduled assessment should be performed.

If a subject inadvertently misses a prescribed tumor evaluation or a technical error prevents the evaluation, the subject may continue treatment until the next scheduled assessment, unless signs of clinical progression are present.

Bilateral Mammary ultrasound

Tumor response will be evaluated during the neoadjuvant period; tumor local progression and recurrence will be evaluated at the end of treatment of withdrawal and during the post treatment following-up.

For each subject, the same method of assessment and the same technique **must** be used to evaluate each breast throughout the entire study. Tumor measurements should be made by the same investigator/radiologist for each patient during the study to the extent that this is feasible.

Scheduling of assessments

Baseline total tumor burden must be assessed within a maximum of 4 weeks before first dose of study drug treatment. Post-baseline assessments are to be performed if clinically indicated during the neoadjuvant and adjuvant periods, at the end of treatment (W56) and once a year during the pot-treatment follow-up.

If there is suspicion of progression or recurrence based on clinical or laboratory findings before the next scheduled assessment, an unscheduled assessment should be performed.

All tumor assessments after baseline will be done within **3 days** of the scheduled visit. If a subject inadvertently misses a prescribed evaluation or a technical error prevents the evaluation, the subject may continue treatment until the next scheduled assessment, unless signs of clinical progression or recurrence are present.

Other imaging assessements

Underlying malignancy will be evaluated by:

- abdominal US + chest-X-ray (+ scanner TAP if abnormal)
- bone scintigraphy will be performed at any time.

For each subject, the same method of assessment and the same technique **must** be used to evaluate each lesion throughout the entire study. Measurements should be made by the same investigator/radiologist for each patient during the study to the extent that this is feasible.

Scheduling of assessments

Baseline assessment of other tumor or metastases must be assessed within a maximum of 4 weeks before first dose of study drug treatment (screening).

Post-baseline abdominal US + chest-X-ray assessments are to be performed at W19, W46, and every 6 months.

Post-baseline bone-scintigraphy assessments are to be performed at W19.

If there is suspicion of underlying malignancy progression based on clinical or laboratory findings before the next scheduled assessment, an unscheduled assessment should be performed.

All tumor assessments after baseline will be done within **3 days** of the scheduled visit. If a subject inadvertently misses a prescribed evaluation or a technical error prevents the evaluation, the subject may continue treatment until the next scheduled assessment, unless signs of clinical progression or recurrence are present.

5.3.6 Breast surgery

The type of breast surgery will be established by the surgeon as conservative or non conservative surgery according to centre practices: axillary procedure (sentinel node or axillary node dissection) and immediate breast reconstruction are allowed.

5.3.7 Performance status

Performance Status (PS) will be measured using the ECOG Performance Status Scale (See Appendix 4).

It is recommended, where possible, that a subject's PS will be assessed by the same person throughout the study.

PS will be assessed at each visit from screening to last study treatment cycle.

5.3.8 Clinical Safety Assessments

The NCI CTC-AE V4.0 will be used to evaluate the clinical safety of the treatment in this study (see Appendix 9).

Subjects will be assessed for adverse events at each clinical visit and as necessary throughout the study.

A complete medical history (including demographics) will be performed at screening. It will include concomitant diseases and treatments, history of previous cancer, and surgical history. A history of breast cancer will also be performed including diagnosis date and TNM clinical staging at diagnosis, HER 2 and hormone receptors status (needle core biopsy only).

Vital signs (height, weight, pulse rate, blood pressure, and body temperature) will be assessed at each visit from screening to last study treatment cycle. A physical examination will be performed at screening and at each visit during the neodajuvant treatment period and at Cycle 16.

An ECG will be performed at screening. LVEF could be assessed by MUGA or echocardiogram at screening; however the same method should be used throughout the whole study (Table 1 and Table 2).

5.4 Laboratory Assessments

The maximum volume of blood loss for laboratory assessments will be approximately 165 mL (except blood samples for translational research studies).

Translational research studies are optional; the maximum volume of blood loss for laboratory assessments will be:

- 60 mL for pharmacokinetics
- 10 mL for immunology
- 5 mL for pharmacogenetics
- 100 mL for angiogenesis study (only for Dijon patients).

For the centralized analyses of the translational research studies, the procedures for the collection, handling and shipping of laboratory samples are specified in Figure 3 and in Appendix 11.

5.4.1 Efficacy Laboratory Assessments

Not applicable.

5.4.2 Safety Laboratory Assessments

Haematology and biochemistry will be done as part of regular safety assessments. Specifically at the times detailed in Table 1 and Table 2:

- Haematology: complete blood count (CBC) with white blood cell differential count, platelet count and haemoglobin;
- Biochemistry: glycaemia, serum creatinine, creatinine clearance, AST, ALT, alkaline phosphatase, GGT, serum bilirubin, LDH;
- Urinalysis for proteinuria (dipstick): at screening with 24 hour urine proteinuria if available and after radiotherapy, to be done only if the previous dipstick for proteinuria $\geq$ 2+;
- Blood Pregnancy Test (HCG-β): at screening for all women of childbearing potential or amenorrheic for the last 24 months, and at each following visit if it is clinically indicated;
- Coagulation monitoring (only for patients receiving a coumarin anticoagulant treatment): INR, TCA.

5.5 Predictive factors studies

In this study, in addition to HER2/RH status, pathological complete response, imaging and scintigraphy results (PET and mammary DCE-US), translational research studies [pharmacokinetics, pharmacogenetics, immunology, and angiogenesis biomarkers

(biology and imaging)] will be conducted in order to establish predictive factors of efficacy and safety.

The main procedures of the predictive factors study are presented in Figure 3.

The rationale, procedures and analysis of HER2/RH status, pathological complete response, scintigraphy and imaging results (PET and mammary DCE-US) are detailed in the core protocol.

The rationale, procedures and analysies of the pharmacokinetics, pharmacogenetics, immunology, and angiogenesis biomarkers (biology and imaging) studies are detailed in Appendix 11.

The maximum volume blood loss for translational research studies assessments will be approximately 225 mL.

Figure 3: Schedule of Assessments for Predictive Factors Studies (neoadjuvant period)

	TREATEMENT ARM	SPECIFIC CONSENT	SAMPLES	CENTRE PREPARATION	CONSERVATION TEMP	SHIPPING SCHEDULE	SHIPPING MANAGER	SHIPPING DESTINATION
TRANSATIONAL RESEARCH STUDIES (optional)								
Study n°1: PK-PD	All Arms	yes	blood T0, T2h (+/- TD21*) (2 x 5ml) C1, C2, C3, C4, C5 and C6	centrifuge and aliquot	-20°C	after the last sampling of each patient	CRAs	Pr Paintaud Tours
Study n°2: Immunologic	All Arms	yes	Blood 2 x 5ml at D1	EDTA	Room temperature	After the sampling of each patient	the centre	Pr Thibault Tours for 1 sample
Study n°3: Pharmacogenetic	All Arms	yes	whole blood 1 x 5ml at D1		-20°C	after the last sampling of each patient	CRAs	Pr Paintaud Tours
Study n°4a: Angiogenesis (biology) (only Dijon patients)	All Arms : C1 to C6	yes **	blood 1 x 25ml before each cycles C1 to C6		Room temperature		the centre	Dr Coudert Dijon
CENTRALIZED REVIEW								
HER2/RH	All patients	no	Tumor at screening	plates	Room temperature	after each patient's sampling	pathologist	Dr Arnould Dijon
pCR	All patients	no	Tumor at baseline and surgery	plates	Room temperature	after the last sampling of each patient	pathologist	Dr Arnould Dijon

*if Cycles delay

** specific information form required

6. INVESTIGATIONAL MEDICINAL PRODUCT

The **investigational medicinal product in this study is bevacizumab** (Avastin®, Roche). The other pharmaceutical treatments are docetaxel (Taxotere®, Sanofi Aventis) and trastuzumab (Herceptin®, Roche).

Bevacizumab will be provided free of charge by Roche. Trastuzumab and docetaxel will be provided by each investigation site.

6.1 Dose and Schedule of Test "Drug" and Comparator(s)

The total number of injections will be as followed:

- 6 injections of docetaxel (C1 to C6, neoadjuvant treatment);
- 18 injections of trastuzumab (C1 to C18, neaoadjuvant and adjuvant treatment);
- For ArmA only, 4 injections of bevacizumab (C3 to C6, neoadjuvant treatment).

Table 3: Description of the dosage regimens of study medication

Drug	Dose	Dosing interval	Planned duration per period
Neoadjuvant treatment *(Cycles 1 – 2)*			
Docetaxel	100 mg/m^2 IV over 60 minutes *(See Section 6.1.3.6 for premedication instructions)*	Day 1 every 3 weeks	For 2 injections
Trastuzumab	8 mg/kg IV loading dose over 90 min	First dose only	
	Subsequent doses: 6 mg/kg IV over 30-60 min	Day1 Every 3 weeks	For 2 injections
Neoadjuvant treatment *(Cycles 3 –7)*			
Bevacizumab (Arm A)	15 mg/kg IV over: 90 minutes – 1st dose* 60 minutes – 2nd dose* 30 minutes – all subsequent doses* Flush infusion line	Day 1 every 3 weeks	For 4 injections
Docetaxel (All Arms)	100 mg/m^2 IV over 60 minutes *(See Section 6.1.3.6 for premedication instructions.)*	Day 1 every 3 weeks	For 4 injections
Trastuzumab (All Arms)	6 mg/kg IV over 30-60 min	Day 1 Every 3 weeks	For 5 injections

Drug	Dose	Dosing interval	Planned duration per period
Adjuvant treatment *(Cycles 8 – 18)*			
Trastuzumab **	6 mg/kg IV over 30-60 min	Day1 Every 3 weeks	For 11 injections
Hormonal therapy (mandatory if positive receptors)	Tamoxifen (not menopausal patient) Anti-aromatases (menopausal patient)	Every day	At investigator's discretion
If one of the treatments is stopped due to toxicity or patient refusal, the other treatments will be continued according to the recommendations in Section 6.1.3.1. *General instructions for delays* * The initial dose of bevacizumab should be delivered over 90 (±15) minutes. If the first infusion is well tolerated and there are no adverse events associated with the infusion (fever and/or shivering), the second infusion may be administered over 60 (±10) minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be administered over 30 (±10) minutes. Bevacizumab may be given before or after chemotherapy administration. ** Trastuzumab treatment will be maintained during ***perioperative period and*** radiotherapy.			

6.1.1 Bevacizumab (Investigational Medicinal Product)

Patients with Δ SUV < 70% will be randomized after the first 2 cycles of neoadjuvant treatment (docetaxel + trastuzumab) (Figure 2). Then, patients in Arm A will receive the following combination therapy: docetaxel + trastuzumab + bevacizumab.

The dose of bevacizumab selected for the neoadjuvant phase of this study is 15mg/kg per IV infusion every 3weeks, for a period of 12 weeks (4 injections, Cycles 3 – 6). The duration of the administration will be as followed:

- 90 minutes IV infusion for the 1st administration; then if well tolerated,
- 60 minutes IV infusion for the 2nd administration; then if well tolerated
- 30 minutes IV infusion for the following doses.

6.1.2 Comparators and concomitant study treatments

6.1.2.1 Pharmaceutical Treatment

DOCETAXEL

Patients included in the study will start neoadjuvant treatment with docetaxel IV (100mg/m² Q3W) for 6 cycles, in all treatment arms.

TRASTUZUMAB

Patients included in the study will start neoadjuvant treatment with trastuzumab IV (8mg/kg loading dose and 6mg/kg subsequent doses, Q3W) for 7 cycles, in all treatment arms.

Three weeks after Day 1 of the last neoadjuvant cycle (Cycle7), patients will be go on with trastuzumab IV (6mg/kg, Q3W) treatment for 11 additional cycles (adjuvant treatment).

HORMONAL THERAPY (mandatory if the patient is hormone receptors positive)

Hormonal therapy (adjuvant) will be introduced after the end of the radiotherapy period (*drug, frequency, duration are left at the investigator's discretion*).

6.1.2.2 Non-Pharmaceutical Treatment

SURGERY

All patients will undergo surgery **after Cycle 7 and between 4 and 5 weeks after the treatment infusion of Cycle 6** (ie: after a delay of 28 to 35 days after the last infusion of bevacizumab for patients of Arm A).

Note: Trastuzumab (6mg/kg) will be maintained during the perioperative period.

RADIOTHERAPY

Radiotherapy will start about **4 to 8 weeks** after the surgery, as soon as the wound has properly healed, and will last around **4 - 6 weeks** depending on the site standard practices. Dose and frequency are left to investigator's discretion and according to site's standard practice.

Note: Trastuzumab (6mg/kg) will be maintained during radiotherapy.

6.1.3 Dose Modifications and Delays

6.1.3.1 General instructions for delays

In case of toxicity requiring the temporary interruption of either bevacizumab or trastuzumab or docetaxel, treatment will be continued with the remaining treatments.

Table 4: General instructions for treatments delays

Docetaxel discontinued	Proceed to bevacizumab and trastuzumab
Docetaxel delayed	**By less than a week (≤ 7 days)**: bevacizumab and trastuzumab administration must also be delayed accordingly. **By more than one week (> 7 days):** bevacizumab and trastuzumab should be administred as soon as possible.

Bevacizumab discontinued	Continue docetaxel and trastuzumab
Bevacizumab delayed	Do not delay the administration of the chemotherapy regimen and trastuzumab No dose reduction of bevacizumab is allowed
Trastuzumab discontinued	Continue docetaxel and bevacizumab (*if applicable*)
Trastuzumab delayed	If the patient misses a dose of trastuzumab **by less than a week (≤ 7 days)**: usual dose of trastuzumab (6 mg/kg) should be given as soon as possible (do not wait until the next planned cycle). Subsequent maintenance trastuzumab doses of 6mg/kg are then given every 3 weeks, according to the previous schedule. **by more than one week (> 7 days):** a re-loading dose of trastuzumab should be given (8mg/kg over 90 minutes). In general, subsequent maintenance trastuzumab doses of 6mg/kg are then given every 3 weeks, starting 3 weeks later. No dose reduction of trastuzumab is allowed.

6.1.3.2 General instructions for Dose modification

Dose modifications due to toxicity are presented below. Toxicity will be graded according to CTCAE version 4.0 criteria (Appendix 9).

The action to be taken in the event of protocol modifications (treatment interruption or dose modification due to an AE) is specific for each treatment. The action taken, the treatment and the follow up will be recorded in the patient source document and the CRF.

For any AE already present at inclusion, dose modifications can be made as a consequence of changes in the toxicity grade, if judged acceptable by the investigator. For example, if a patient presents grade 1 asthenia at inclusion which becomes grade 2 during the course of the study, this would be considered as a worsening of grade and treated as grade 1 for dose modifications.

For toxicities that the investigator considers will not progress to serious or life-threatening adverse events (alopecia, taste-bud changes, etc.), the treatment will be continued and will remain unchanged. Similarly, no dose modification will be made for anemia (non hemolytic) since it can easily be treated by transfusions.

When several toxicities of different grades and severities appear at the same time, the dose modifications will be made according to the greatest applicable dose reduction.

If the investigator judges that the toxicity is due to a single product (hypertension and proteinuria due to bevacizumab), the doses of other products will not be modified.

During this study, there will be no dose adjustments of bevacizumab, except if the patient's weight varies by more than 10%, in which case the dose will be recalculated. The administration of bevacizumab must be temporarily or definitively interrupted in the event of certain adverse events such as: hemorrhage, thromboembolic events (arterial or venous), hypertension, severe proteinuria, gastrointestinal perforations, congestive heart failure, wound healing complications, intra abdominal fistules or abscesses, depending on their nature and their grade and according to the recommendations in Table 6).

Dose modifications for isolated cases of abnormal blood parameters will be based on the value of the parameter at the cycle start. Since no blood tests are planned during the course of a cycle, no nadir values will be taken.

If toxicity requires more than 3 weeks prolongation of the rest period for ALL treatments, the patient will be withdrawn from the study treatment period for toxicity.

Hematological toxicity

Treatment should be maintained if neutrophil level $\geq$ 1 500/mm^3 and platelet level $\geq$ 100 000/mm^3. If these conditions are not met, **chemotherapy could be delayed for a maximum of 21 days.**

Complete blood count and platelets levels will be analysed in case of fever $\geq$ 38.5°C and controlled in a maximum of 7 days later.

If the treatment is delayed for neutropenia, the use of growth factors is allowed.

Cutaneous toxicity

In case of grade 3 cutaneous toxicity, docetaxel will be delayed for a maximum of 3 weeks until grade $\leq$ 2.

Grade 4: discontinue docetaxel.

Any other non hematological toxicity related to chemotherapy

Any other type of grade 3 or 4 non hematological toxicity should return to normal or grade 1 or 2 before starting the administration of the following cycle.

6.1.3.3 Bevacizumab Dose Adjustment and Delays due to Toxicity

Bevacizumab will be administered as 15 mg/kg Q3w regimen schedule (for patients of Arm A only). Median treatment duration with bevacizumab is expected to be 12 weeks (4 Cycles).

The dose for bevacizumab will be calculated as milligrams per kilogram body weight (mg/kg). The subject's weight at screening will be used to determine the dose of bevacizumab to be used for the duration of the treatment. If the subject's weight changes by $\geq$ 10% (compared to the weight at the 1st administration) during the neoadjuvant treatment period, the dose of bevacizumab will be recalculated.

There will be no bevacizumab dose reductions for toxicity. Missed doses of bevacizumab will not be replaced.

In case of serious bevacizumab-related toxicity, bevacizumab treatment will either be temporarily or permanently suspended. If bevacizumab treatment is permanently discontinued, but chemotherapy is not interrupted, the patient will continue the study as planned (have surgery, radiotherapy and then enter the follow-up phase of the study).

As described below, bevacizumab treatment may be either temporarily or permanently suspended in the case of hypertension, proteinuria, thrombosis/embolism, haemorrhage, CHF or wound healing complications in addition to any other serious bevacizumab-related toxicity (grade 3 or 4).
Bevacizumab should be temporarily withheld in the event of febrile grade 4 neutropenia and/or grade 4 thrombocytopenia (regardless of the relationship to treatment), since these conditions are predisposing factors for an increased bleeding tendency. In general, appropriate management for grade 3 or 4 bevacizumab-related events is described in Table 5.

Table 5 – Management of grade 3 or 4 bevacizumab-related AEs

First occurrence	Hold bevacizumab until toxicity has improved to ≤ grade 1
Second occurrence	Permanently discontinue bevacizumab treatment

In addition, bevacizumab treatment should be permanently discontinued in patients experiencing any of the following events:

- Reversible Posterior Leucoencephalopathy Syndrome (RPLS)
- Grade 3/4 haemorrhagic/bleeding events
- Any grade of arterial thromboembolism
- Grade 4 hypertension (hypertensive crisis)
- Grade 4 proteinuria (nephrotic syndrome)
- Grade 3/4 left ventricular dysfunction (CHF)
- Any grade of gastrointestinal perforation
- Any grade of tracheo-esophageal fistula
- Any grade 4 non-gastrointestinal fistula
- Any grade of hypersensitivity/allergic reactions related to bevacizumab.

Furthermore:

- Toxicity will be graded according to the NCI CTC-AE version 4.0 (see Appendix 9).
- Specific instructions on the management of hypertension, proteinuria (for more details, see Appendix 7), thrombosis/embolism, hemorrhage and other events attributable to bevacizumab are provided in Table 7.
- Instructions regarding cardiac toxicity (congestive heart failure) are described in Section 6.1.3.5.

- If bevacizumab must be discontinued due to ***bevacizumab-related toxicity*** before completion of the neoadjuvant treatment, docetaxel and trastuzumab should be continued as per protocol.
- If alternative (non-protocol) chemotherapy is given at any time, bevacizumab must be discontinued.
- Depending on the toxicity grade, bevacizumab administration should be modified as summarized in Table 7.

Table 6: Treatment delays and instructions for bevacizumab-related adverse events

Adverse Event	Grade CTCAE v4.0	Action to be Taken
Infusion related or Allergic reaction/ Acute hypersensitivity reaction (e.g., fever, rash, urticaria, bronchospasm)	1, 2, or 3	- *If infusion-related or allergic reactions (cytokine release syndrome/acute infusion reaction or allergic reaction/hypersensitivity such as fever, rash, urticaria or bronchospasm) occur, pre-meds should be given with the next dose*, but the infusion time may not be ↓ for the subsequent infusion. If the next dose is well-tolerated with pre-meds, the subsequent infusion time may be ↓ by 30 ± 10 min. as long as pre-meds continue to be used. If infusion-related AEs occur with the 60-min. infusion, all subsequent doses should be given over 90 ± 15 min. (with pre-meds). If infusion-related AEs occur with the 30-min. infusion, all subsequent doses should be given over 60 ± 10 min. (with pre-meds). - *For patients with grade 3 reactions*, the bevacizumab infusion should be stopped and not re-started on that day. At the physician's discretion, bevacizumab may be permanently discontinued or re-instituted with pre-medications and at a rate of 90 ± 15 minutes. If the reaction occurred at the 90-minute rate, initially challenge at a slower infusion rate and gradually increase to 90 min. When bevacizumab is re-instituted, the patient should be monitored per physician's usual practice for duration comparable to duration of reaction.
	4	Permanently discontinue bevacizumab.
Hypertension	1	Bevacizumab may be continued if asymptomatic, transient (<24 hrs) BP increase by >20 mmHg (diastolic) or to >150/100 mmHg (if previously within normal limits).
	2	Hold bevacizumab if recurrent or persistent (>24 hr) or symptomatic increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal limits. Start antihypertensive therapy. Once BP is <150/100 mmHg, patients may continue bevacizumab therapy.
	3	If hypertension requires more than one anti-hypertensive drug or more intensive therapy than previously, Hold bevacizumab for persistent or symptomatic hypertension and discontinue permanently if hypertension is not controlled according to Investigator judgment.
	4	Permanently discontinue bevacizumab.
Hemorrhage	3 or 4	Permanently discontinue bevacizumab. ***(Note: Bevacizumab must also be permanently discontinued for grade 2 pulmonary or CNS hemorrhage.)*** If hemorrhagic complications occur in patients on full dose anticoagulation therapy, permanently discontinue bevacizumab treatment and follow guidelines of the institution. Standard procedures such as antagonisation with protamine or vitamin K, infusion of vitamin K dependent factors or insertion of a vena cava filter should be considered dependent on the severity of the bleeding and thrombotic events and the organ affected.

Adverse Event	Grade CTCAE v4.0	Action to be Taken
Thrombosis/embolism-venous (including vascular access device)	2, 3, or 4	Arterial Thromboembolism: If a patient experiences *any grade* of arterial thromboembolism during the study treatment period, bevacizumab should be discontinued permanently. Venous Thromboembolism: Patients experiencing a grade 4 thrombosis must be discontinued from the study. If a patient experiences a grade 3 venous thromboembolism, bevacizumab must be withheld for 3 weeks. Bevacizumab may be resumed during the period of therapeutic-dose anticoagulant therapy.
	4	Permanently discontinue bevacizumab.
Visceral or peripheral arterial ischemia	2[a,b], 3, or 4	Permanently discontinue bevacizumab.
CNS ischemia	3 or 4	Permanently discontinue bevacizumab.
Signs of Reversible Posterior Leuco encephalopathy Syndrome (RPLS)	≥ 1	For clinical features suggestive of RPLS, hold bevacizumab and obtain MRI. If RPLS is diagnosed ***or*** if ANY symptoms were grade 4, permanently D/C bevacizumab. If RPLS is not diagnosed, bevacizumab may be resumed when presenting symptoms ≤ grade 1 ***and*** blood pressure < 150/90 mmHg.
GI perforation including GI leak and GI fistula	≥ 1	Permanently discontinue bevacizumab.
Non-GI perforation including non-GI fistula	≥ 1	Permanently discontinue bevacizumab.
Intra-abdominal abscess[c]	3	Hold bevacizumab until resolved.
	4	Permanently discontinue bevacizumab.
Wound healing complications *(not applicable for breast surgery)*	1	Hold bevacizumab for at least 3 weeks. If, in the physician's opinion, substantial healing has taken place within 1-3 months, bevacizumab may be resumed. If wound dehiscence recurs, permanently discontinue bevacizumab.
	2, 3, or 4	Permanently discontinue bevacizumab.
Proteinuria[e]	3	Hold bevacizumab until proteinuria improves to ≤ grade 2. Re-check every 3-6 weeks. If proteinuria does not improve to ≤ grade 2 within 6 weeks, permanently discontinue bevacizumab.
	4	Permanently discontinue bevacizumab.
Other clinically significant adverse events[f]	3	Hold until AE has resolved to ≤ grade 1.
	4	Permanently discontinue bevacizumab.

a New grade 2 events. (Therapy may be continued for grade 2 conditions present at baseline)
b Pts. who develop brief, reversible, exercise-induced claudication (grade 2) not attributable to arterial thromboembolic events may continue on study.
c Refer to grading criteria listed for the appropriate AE in the Infection Section of the CTCAE v 4.0.
d Refer to Dermatology/Skin Section of the CTCAE v 4.0.
e CTCAE v4.0 criteria for grading proteinuria in g/24 hours will be estimated by use of dipstick testing; ***24-hour urine collection is required for ≥ grade 2 (dipstick 2+ to 3+).*** For more details, see appendix 6
f Determination of "clinically significant" is at the physician's discretion and applies to those ***AEs that are not clearly associated with chemotherapy or with trastuzumab and could be related to bevacizumab.***

6.1.3.4 Trastuzumab Dose Adjustment and Delays due to Toxicity

The most serious adverse reactions caused by trastuzumab include congestive heart failure and hypersensitivity reactions including anaphylaxis, and infusion reactions.

Congestive Heart Failure (CHF)

Trastuzumab will be discontinued in any patient who develops clinical signs and symptoms suggesting CHF. The CHF should be treated and followed according to standard medical practice.

For all patients experiencing a cardiac event, the frequency of cardiac monitoring (LVEF assessment) will be increased and must be performed at all patient visits up to year 2 and then every 3 months for a total of 2 years following the initial diagnosis of CHF.

Asymptomatic and Mildly Symptomatic Drops in LVEF

Confirmed significant asymptomatic (NYHA class I) and mildly symptomatic (NYHA class II) drops in LVEF are secondary cardiac events. At the present time, there are inadequate data available to assess the prognostic significance of asymptomatic drops of LVEF. However, to ensure the safety of patients in the trial, trastuzumab must be discontinued in all patients, for whom a drop of LVEF to below 45% is documented and confirmed with a subsequent repeat assessment approximately 3 weeks after the first assessment, using the same assessment method. The frequency of cardiac monitoring (LVEF assessment) will be increased and will be performed at all patient visits up to year 2 and then every 3 months for a total of 2 years following the initial diagnosis of CHF (NYHA class I or II). If trastuzumab is held for 2 consecutive cycles or 3 intermittent cycles, it must be discontinued (see Figure 4). The cardiac monitoring schedule (LVEF assessment) will be performed at all every 3 months under treatment (approximately 52 weeks), 6 months after last trastuzumab injection and then once per year until the end of the study (5 years after the inclusion of the patient).

Infusion Reactions and hypersensitivity

Serious adverse reactions to trastuzumab infusion that have been reported infrequently include dyspnea, hypotension, wheezing, bronchospasm, asthma tachycardia, reduced oxygen saturation, anaphylaxis, respiratory distress, urticaria and angioedema. The majority of these events occur during or within 2.5 hours of the start of the first infusion. Should an infusion reaction occur the Herceptin® infusion should be discontinued and the patient monitored until resolution of any observed symptoms. The majority of patients experienced resolution of symptoms and subsequently received further infusions of trastuzumab. Serious reactions have been treated successfully with supportive therapy such as oxygen, beta-agonists, and corticosteroids. In rare cases, these reactions are associated with a clinical course culminating in a fatal outcome. Patients with dyspnea at rest due to co-morbidities may be at increased risk of a fatal infusion reaction. Therefore, these patients should not be treated with trastuzumab.

6.1.3.5 Management of Congestive Heart Failure (CHF) during Bevacizumab and Trastuzumab treatment – LVEF assessment

Note: Trastuzumab and bevacizumab **must** be discontinued in patients who have a **symptomatic** decrease in LVEF.

Instructions for symptomatic decrease in LVEF:

- *Grade 3 CHF:* Patients should be monitored for signs and symptoms of CHF i.e., dyspnea, tachycardia, cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, etc. **If the patient develops any of these signs and symptoms, trastuzumab and bevacizumab must be held.**

The investigator must confirm the diagnosis of CHF either with an echocardiogram or MUGA scan. **Once the diagnosis of CHF is confirmed, trastuzumab and bevacizumab must be permanently discontinued**, and all reports must be submitted with the cardiac event report form and the LVEF assessment form within 21 days of the LVEF assessment. The protocol-specified schedule for obtaining LVEF assessment should be followed even after the discontinuation of protocol therapy or occurrence of a cardiac event.

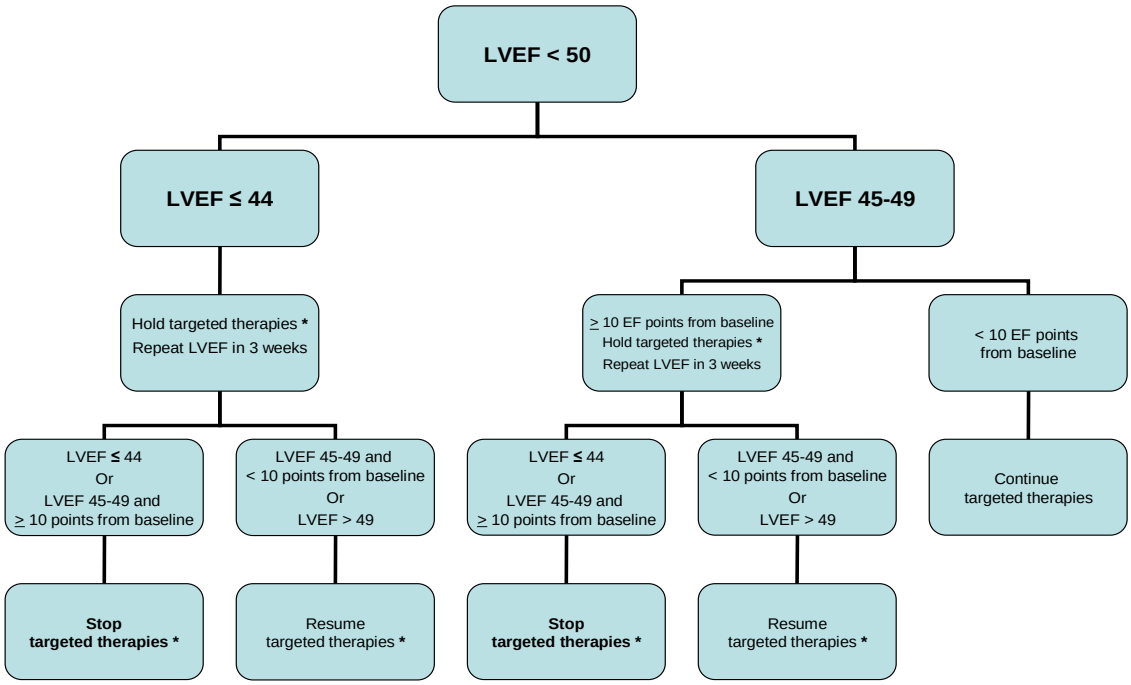
- *Grade 4 CHF (severe refractory CHF or requiring intubation)*: **Discontinue trastuzumab and bevacizumab.**

Instructions for asymptomatic decrease in LVEF:

- In case of asymptomatic decrease in LVEF > or equal to 50% following LVEF assessment during study therapy, targeted therapies* has to be continued.
- In case of asymptomatic decrease in LVEF < 50% following LVEF assessment during study therapy, instructions regarding the targeted therapies* are provided in Figure 4.

** targeted therapies : bevacizumab and trastuzumab*

Figure 4 Bevacizumab and trastuzumab management based on LVEF assessments



** targeted therapies : bevacizumab and trastuzumab*

- Trastuzumab and bevacizumab must be discontinued when two consecutive "hold therapy" categories occur.
- Bevacizumab must be discontinued if bevacizumab is held <u>two consecutive or two intermittent</u> times.
- Trastuzumab must be discontinued when <u>three intermittent</u> "hold therapy" categories occur or two consecutive “hold therapy” categories occur.

6.1.3.6 Docetaxel Dose Adjustment and Delays due to Toxicity

The most serious adverse reactions caused by docetaxel are hematological and acute hypersensitivity reactions.

<u>Hematological reactions:</u>

- Recommendations based on neutropenia:

Patients should not be treated with docetaxel if neutrophils are < 1 500 / mm^3.
Patients who experience either febrile neutropenia, neutrophils < 500 / mm^3 for more than 1 week during docetaxel therapy should have the dosage adjusted from 100 mg/m² to 75 mg/m² and/or from 75 to 60 mg/m². If the patient continues to experience these

reactions at 60 mg/m², the treatment should be discontinued. G-CSF may be used to mitigate the risk of hematological toxicities.

- Recommendations based on thrombocytopenia:

Patients should not be treated with docetaxel if platelets are < 100 000 / mm^3.

- Patients who experience platelet level between 25 and 100 000 / mm^3 during docetaxel therapy, should have the following cycle delayed (7 days)
- Patients who experience platelet level platelet level < 25 0000 / mm^3, should have the dosage adjusted from 100 mg/m² to 75 mg/m² and/or from 75 to 50 mg/m². If the patient continues to experience this reaction at 50 mg/m², the treatment should be discontinued.

Acute hypersensitivity reactions:

Recommendations based on the intensity of the hypersensitivity reaction:
Light: Local cutaneous reactions (pruritus, rash, flush), slow the infusion rate, close monitoring until resolution, then continue the infusion at the initial infusion rate.
Moderate: any reaction between light and severe: pruritus, flush, generalized rash, dyspnea, hypotension > 50 mmHg: stop the infusion of docetaxel, antihistaminic agents and steroids. Following the complete disappearance of the symptoms, continue the infusion at the initial infusion rate. During the following cycles, increase the premedication dose of steroids and antihistaminic agents.
Severe: bronchospasms, generalized urticaria, hypotension < 80mmHg, and angioedema: stop the infusion of docetaxel, antihistamines agents and steroids. Use epinephrine or bronchodilatator and/or macromolecules if necessary. Following the total disappearance of the symptoms docetaxel can be restarted in the following 24h, if clinically possible after an initial premedication. For the following cycles administer prednisolone at a dose of 100 mg PO at H-24, H-17, H-13, H -7, H-01; cetirizine 10 mg at H-13 and polaramine 50 mg IV 1 hour before docetaxel. If there is another severe reaction, the patient will be withdrawn from the study.
In case of **edematous syndrome**, the decision to continue or discontinue the treatment will be left to the clinical judgment of the investigator based on clinical information and response to treatment. The appearance of a pleural effusion or ascites will need to be carefully assessed in order to eliminate a progressive disease.

Extravasation:

No severe extravasation reactions have been described with docetaxel. In case of extravasation, the investigator will apply the standard procedure of the center.

Alopecia:

Alopocia will not be considered as a toxicity requiring treatment modifications.

6.1.3.7 Radiotherapy Dose Adjustment and Delays due to Toxicity

Treatment modifications in the event of an AE during radiotherapy will be managed according to the center's standard procedures.

6.2 Preparation and Administration of Test "Drug" and Comparator(s)

6.2.1.1 Bevacizumab

Bevacizumab will be administered as an IV infusion initially over a 90-minute period. If the first infusion is well tolerated, the second infusion may be delivered over a 60-minute period. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over a 30-minute period.
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Bevacizumab vials should be stored in a refrigerator (2°C – 8°C) and kept in the outer carton in order to protect from light.

The necessary amount of bevacizumab will be withdrawn from the vial(s) and diluted to the required administration volume in pyrogen-free 0.9% sodium chloride solution for injection using aseptic techniques. The concentration of the final bevacizumab solution should be kept within the range of 1.4-16.5 mg/ml.
Any unused portion left in a vial should be discarded as the product contains no preservatives. Bevacizumab infusions should not be administered or mixed with dextrose or glucose solutions. Dose of bevacizumab can be given before or after docetaxel.
It is important to flush the lines with saline solution after the bevacizumab infusion.

Do not administer as an IV push or bolus

When extravasation of bevacizumab occurs during an infusion, the following actions are recommanded:

- Discontinue the infusion,
- Treat the extravasation according to institutional guidelines for extravasation of a non-caustic agent,
- If a significant volume of bevacizumab remains in the infusion bag, restart at a more proximal site ipsilaterally or on the contralateral limb.

6.2.1.2 Trastuzumab

Trastuzumab will be administered as a 90-minute IV infusion.

Patients should be observed for at least six hours after the start of the first infusion and for two hours after the start of the subsequent infusions for symptoms like fever and chills or other infusion-related symptoms. Interruption of the infusion may help control such symptoms. Te infusion may be resumed when symptoms abate.
If the initial loading dose was well tolerated, the subsequent doses can be administered as a 30-minute infusion. Emergency equipment must be available.

Trastuzumab vials should be stored in a refrigerator (2°C – 8°C).
Appropriate aseptic technique should be used. Each vial of trastuzumab is reconstituted with 7.2 ml of sterile water for injections. Use of other reconstitution solvents should be

avoided. This yields a 7.4 ml solution for single-dose use, containing approximately 21 mg/ml of trastuzumab. A volume overage of 4% ensures that the labelled dose of 150 mg can be withdrawn from each vial.

Instructions for Reconstitution:

- Using a sterile syringe, slowly inject 7.2 ml of sterile water for injections in the vial containing the lyophilised trastuzumab, directing the stream into the lyophilised cake;
- Swirl vial gently to aid reconstitution. DO NOT SHAKE!

Determine the volume of the solution required, based on a loading dose of 8 mg trastuzumab/kg body weight, or a subsequent 3-weekly dose of 6 mg trastuzumab/kg body weight:

$$\text{Volume (ml)} = \frac{\text{Body weight (kg) x dose (8 mg/kg for loading or 6 mg/kg for maintenance)}}{\text{21 (mg/ml, concentration of reconstituted solution)}}$$

The appropriate amount of solution should be withdrawn from the vial and added to an infusion bag containing 250 ml of 0.9% sodium chloride solution. Do not use with glucose-containing solutions.

Parenteral solutions should be inspected visually for particulates and discoloration prior to administration. Once the infusion is prepared it should be administered immediately. If diluted aseptically, it may be stored for 24 hours (do not store above 30°C).

More details can be found in the SPC of trastuzumab.

6.2.1.3 Docetaxel

Docetaxel will be administered as a 60-minute IV infusion.

All patients should be premedicated with oral corticosteroids such as dexamethasone 16 mg per day (e.g., 8 mg BID) for 3 days starting 1 day prior to decetaxel administration in order to reduce the incidence and severity of fluid retention as well as the severity of hypersensitivity reactions.

Docetaxel vials should be stored between 2 and 25°C. If the vials are stored under refrigeration, allow the appropriate number of vials of docetaxel Injection Concentrate and diluent (13% ethanol in water for injection) vials to stand at room temperature for approximately 5 minutes.

Docetaxel Injection Concentrate requires two dilutions prior to administration:

- Initial Diluted Solution

Aseptically withdraw the entire contents of the appropriate diluent vial (approximately 1.8 mL for docetaxel 20 mg and approximately 7.1 mL for docetaxel 80 mg) into a

syringe by partially inverting the vial, and transfer it to the appropriate vial of docetaxel Injection Concentrate. If the procedure is followed as described, an initial diluted solution of 10 mg docetaxel/mL will result.

The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.

- Final Dilution for Infusion

Aseptically withdraw the required amount of initial diluted docetaxel solution (10 mg/mL) with a calibrated syringe and inject into a 250 mL infusion bag or bottle of either 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 to 0.74 mg/mL.

If a dose greater than 200 mg of docetaxel is required, use a larger volume of the infusion vehicle so that a concentration of 0.74 mg/mL docetaxel is not exceeded

As with all parenteral products, docetaxel should be inspected visually for particulate matter or discoloration prior to administration whenever the solution and container permit. If the docetaxel initial diluted solution or final dilution for intravenous infusion is not clear or appears to have precipitation, these should be discarded.

More details can be found in the SPC of docetaxel.

6.3 Formulation, Packaging and Labeling

Bevacizumab (RO 487-6646) will be provided as single use 400 mg (16 mL) and 100 mg (4mL) vials containing a 25 mg/mL concentrate solution for intravenous (i.v.) infusion (trehalose dehydrate, sodium phosphate, polysorbate 20, water for injections, clear to slightly opalescent, colorless to pale brown liquid).

Bevacizumab is presented in colourless glass vials with a butyl rubber stopper with aluminium seal and plastic flip-off disk.

Instructions for Use, Handling and Disposal

Bevacizumab should be prepared by a healthcare professional using aseptic technique. The necessary amount of bevacizumab is to be withdrawn for a dose of 15 mg/kg of body weight and is to be diluted in a total volume of 100 mL of 0.9% sodium chloride injection. Any unused portion left in a vial is to be discarded, as the product contains no preservatives. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Special Precautions for Storage

Vials are to be stored in a refrigerator (2°C – 8°C). Vials are to be kept in the outer carton due to light sensitivity.

DO NOT FREEZE. DO NOT SHAKE.

Bevacizumab does not contain any antimicrobial preservative; therefore, care must be taken to ensure the sterility of the prepared solution.

Chemical and physical in-use stability has been demonstrated for 48 hours at 22-30°C in 0.9% sodium chloride solution. From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user and would normally be no longer than 24 hours at 2-8°C, unless dilution has taken place in controlled and validated aseptic conditions.

Incompatibilities
No incompatibilities between bevacizumab and polyvinyl chloride or polyolefin bags have been observed. Concentration-dependent changes in the ion exchange chromatography profile were observed when bevacizumab was diluted with dextrose solutions (5%). Therefore, bevacizumab should not be administered or mixed with dextrose or glucose solutions.

Stability
Bevacizumab should not be used after the retest date shown on the pack.

The labelling of bevacizumab will be in accordance with all local legal requirements and conducted according to Good Manufacturing Practice.

Bevacizumab is not specifically conditioned per patient.
The vials will be labelled according to current legal requirements.

6.4 Blinding and Unblinding

Not applicable study is open label

6.5 Assessment of Compliance

The patient should be encouraged to come to the hospital or outpatient clinic every 21 days on the same day of the week for administration of the required treatments.

Accountability and subject compliance will be assessed by maintaining adequate "drug dispensing" and return records.

Accurate records must be kept for each study drug provided by the sponsor. These records must contain the following information:

- documentation of drug shipments received from the sponsor (date received and quantity)
- disposition of unused study drug not dispensed to patient

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the subject to whom the study medication was dispensed
- the date(s), quantity of the study medication dispensed to the subject
- the date(s) and quantity of the study medication returned by the subject
- Date and dose of radiotherapy delivered (a maximum delay of 1 week is acceptable).

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and copies of the dispensing & inventory logs, must be returned to the Roche Monitor at the end of the study.

6.6 Destruction of Study "Drug"

Bevacizumab will not be destroyed by the investigator center after dispensation but will be accounted for and returned to the sponsor.

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons e.g., cytotoxicity. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity (batch numbers or subject numbers) of investigational product(s) destroyed
- Quantity of investigational product(s) destroyed
- Date of destruction (date discarded in designated hazardous container for destruction)
- Method of destruction (the site must provide the sponsor with documentation of their institutional policy and procedures for handling and disposing of hazardous drugs)
- Name and signature of responsible person who discarded the investigational product in a hazardous container for destruction

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 Adverse Events and Laboratory Abnormalities

7.1.1 Clinical AEs

Per the International Conference of Harmonisation [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

7.1.1.1 Intensity

Intensity of all adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events v 4.0 (CTCAE) on a five-point scale (Grade 1 to 5) and reported in detail on the CRF (see Appendix 9).

Cardiac safety will be based on NYHA classification (see Appendix 5).

Adverse events not listed on the CTCAE should be graded as follows:

CTC Grade	Equivalent To:	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject
Grade 3	Severe	Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.
Grade 4	Life threatening/disabling	An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.
Grade 5	Death	AE resulting in death

7.1.1.2 Drug – Adverse Event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

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

The following criteria should be considered in order to assess the relationship as **Yes:**

- Reasonable temporal association with drug administration
- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in death; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;

- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The study will comply with all local regulatory requirements and adhere to the full requirements of the **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** (see Appendix 1).

7.1.1.4 Progression of Underlying Malignancy

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria ***(see Appendix 10)***, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

7.1.2 Treatment and Follow-up of AEs

7.1.2.1 Adverse event reported during Treatment Period

After treatment period continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2

- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow until 28 days after last study dose

The final outcome of each adverse event must be recorded on the CRF.

7.1.2.2 Adverse event reported during Follow-up Period

After follow-up period continue to follow up AEs as follows:

Severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

The final outcome of each adverse event must be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will be recorded on the laboratory results pages of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e. meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed until they have returned to the normal range,

baseline value and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 Handling of Safety Parameters

7.2.1 Reporting of Adverse Events

Any AE occurring during the study treatment period (neoadjuvant and adjuvant therapy) must be reported.

7.2.2 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), regardless of the treatment arm, must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting).

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 28 days after the last dose of study medication.

The definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** will be adhered to. Complete information can be found in Appendix 1.

7.2.3 Reporting of Adverse Events of Special Interest

In this study, "AEs of Special Interest" will include the following AEs (serious or non-serious):

- neutropenia,
- febrile neutropenia,
- wound healing complications,
- proteinuria,
- bleeding/haemorrhage,
- hypertension,
- thromboembolic events (venous or arterial),
- congestive heart failure,
- gastrointestinal perforation,
- fistulae,
- Reversible Posterior Leucoencephalopathy Syndrome (RPLS)

These AEs must be recorded in the CRF.

All adverse events of special interest, whatever the severity and cause, must be reported up to 6 months after the last dose of the study treatment and must be followed up until resolution or return to baseline status.

7.2.4 Pregnancy

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor. The investigator should counsel the subject, discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Pregnancies occurring up to 6 months after the completion of the test "drug" must also be reported to the investigator. Monitoring of the patient should continue until conclusion of the pregnancy.

7.3 Warnings and Precautions

Bevacizumab, trastuzumab and docetaxel should only be initiated under the supervision of a physician experienced in the treatment of cancer. Please refer to the Investigators' Brochure as the primary source of safety information.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

8.1 Primary and Secondary Study Variables

8.1.1 Primary Variable

The primary efficacy endpoint is determined by pathological complete response (pCR) in surgical specimens of mammary tissue and lymph nodes, according to Chevallier's classification (see Appendix 8) and reviewed by an independent Committee (Tumor Histology Independent Review Panel, see Section 10.2).

Table 7: Chevallier's criteria

Grade 1: Disappearance of All Tumors Either in the Breast or in the Nodes	Grade 2: Persistence of Carcinoma In Situ in the Breast Only. No Nodal Invasion	Grade 3: Presence of Invasive Carcinoma With Stromal Alteration	Grade 4: Presence of Invasive Carcinoma Without Modification

The pCR is defined as the absence of invasive cancer cells in both the primary site and lymph nodes (grade 1 and 2).

8.1.2 Secondary Variables

The secondary efficacy variables are pCR according to Chevallier's criteria (according to local procedures) and Sataloff's criteria (review by an independent Committee), ultrasound response (before surgery), rate of conservative surgery and local relapse-, disease-, distant disease-free survival, overall survival, and predictive factors of efficacy.

pCR (Sataloff's criteria): evaluation in surgical specimens of mammary tissue and lymph nodes, according to Sataloff's classification (see Appendix 8) and reviewed by an independent Committee (Tumor Histology Independent Review Panel, see Section 10.2)

Tumor (T)

TA: Total or near total therapeutic effect	**TB: Subjectively >50% therapeutic effect but less than total or near total**	**TC: <50% therapeutic effect but effect evident**	**TD: No response**

Node (N)

NA: No nodal metastasis and evidence of therapeutic effect	**NB: No nodal metastasis or therapeutic effect**	**NC: Nodal metastasis present with evidence of therapeutic effect**	**ND: Nodal metastasis present without evidence of therapeutic effect**

pCR is defined as tumor A (TA)/node A (NA) or TA/NB.

Ultrasound Response (UR): a complete response includes the disappearance of all measurable and assessable disease (based on RECIST criteria without confirmation, ***see Appendix 10***), with no new lesion.

Rate of conservative surgery: proportion of patients who undergo conservative surgery after the neoadjuvant treatment.

Local Relapse-Free Interval (LRFI): time to local recurrence following first administration of neoadjuvant treatment, local recurrence in the ipsilateral or controlateral breast following lumpectomy.

Disease-Free Survival (DFS): DFS is defined as time from first administration of neoadjuvant treatment to local recurrence, local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, distant recurrence, contralateral breast cancer, second primary cancer (other than squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, colon carcinoma in situ, or lobular carcinoma in situ of the breast), or death from any cause.

Distant Disease-Free Interval (DDFI): DDFI is defined as time to distant recurrence following first administration of neoadjuvant treatment.

Overall survival (OS): OS is defined from first administration of neoadjuvant treatment to death any cause. For patients alive at study end (premature discontinuation or final visit), censoring will be the last known date alive.

Other assessments (predictive factors of efficacy and safety): pharmacokinetics, pharmacogenetics, immunology, biomarkers (CTC, CEC) and imaging.

8.1.3 Independent Review Committee

The Tumor Histology Independent Review Panel (see Section 10.2) will review surgical specimens in a blinded fashion to assess the rate of pCR according to Chevallier's and Sataloff's classification.

8.1.4 Safety

Safety of the treatment will be evaluated by adverse events, laboratory tests, vital signs, and performance status, according to CTCAE v4.0 (See Appendix 9). Cardiac safety will be based on NYHA classification (see Appendix 5).

8.2 Statistical and Analytical Methods

8.2.1 Statistical Model

The statistical analysis will performed by a contract research organization (CRO) under the supervision of ROCHE biostatistician using SAS Software release 9.1. A statistical analysis plan will be written before database lock.

The type I (α) error will be 5% (two-sided).

Quantitative data will be summarised by the following descriptive statistics: number of data available, number of missing data, mean, standard deviation, first quartile (Q1), median, third quartile (Q3), minimum and maximum.

Qualitative data will be summarised by the following descriptive statistics: number of data available, number of missing data, frequency and percentage for each modality.

8.2.1.1 Primary Variables

The primary efficacy endpoint is determined by pathological complete response in surgical specimens of mammary tissue and lymph nodes, according to Chevallier's classification (see Section 8.1.1) and reviewed by an independent Committee (Tumor Histology Independent Review Panel, see Section 10.2). For this analysis, missing values on primary endpoint will be considered as failure.

If 11 or less pathological complete responses are observed in the docetaxel + trastuzumab + bevacizumab Arm (among the 48 randomized patients of Arm A), then the combination will be deemed ineffective. If 12 or more pathological complete responses are observed in the docetaxel + trastuzumab + bevacizumab Arm, then the association will be deemed effective.

The pCR rate in all arms will be described with its 95% confidence interval.

A sensitivity analysis will be performed without replacing missing values.

8.2.1.2 Secondary Variables

Analyses will be descriptive.

Pathological complete response rate in surgical specimens of mammary tissue and lymph nodes, according to Chevallier's classification review by the local pathologist and according to Sataloff's classification reviewed by an independent Committee will be described with their 95% confidence interval.

Ultrasound response rate will be described by treatment during the neodajuvant treatment course and **rate of conservative surgery** will be described by treatment.

Local Relapse-Free Interval (LRFI): LRFI is defined as time to local recurrence following first administration of neoadjuvant treatment, local recurrence in the ipsilateral or controlateral breast following lumpectomy.

For patients without any local recurrence in the ipsilateral or controlateral breast following lumpectomy, censoring date will be the first date of regional occurrence, occurrence of distant metastases, second primary cancer, death without evidence of recurrence, last follow-up visit or last contact.

Event	Censoring decision	Event or censored date
Local recurrence, controlateral cancer	Not censored	First date
Regional recurrence, distant metastases, second primary cancer, death without evidence of recurrence	Censored	First date
Lost to follow-up	Censored	Date of last assessment without event
Study ended without local recurrence or controlateral cancer	Censored	Date of last assessment without event

Disease-Free Survival (DFS): DFS is defined as time from first administration of neoadjuvant treatment to local recurrence, local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, occurrence of distant metastases, controlateral breast cancer, second primary cancer (other than squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, colon carcinoma in situ, or lobular carcinoma in situ of the breast), or death from any cause.

For patients without any local recurrence, local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, distant recurrence, controlateral breast cancer, second primary cancer or death from any cause, censoring date will be the date of last follow-up visit.

Event	Censoring decision	Event or censored date
Local or regional recurrence, distant metastases, second primary cancer, controlateral cancer, death	Not censored	First date
Lost to follow-up	Censored	Date of last assessment without event
Study ended without local or regional recurrence, distant metastases, second primary cancer, controlateral cancer, death	Censored	Date of last assessment without event

Distant Disease-Free Interval (DDFI): DDFI is defined as time to distant recurrence following first administration of neoadjuvant treatment.

For patients without any distant metastases, censoring date will be the first date of local or regional recurrence, second primary cancer, controlateral cancer, death from any cause, last follow-up visit.

Event	Censoring decision	Event or censored date
Distant metastases	Not censored	Date of first tumor assessment or examination showing distant metastases

Event	Censoring decision	Event or censored date
Local or regional recurrence, second primary cancer, controlateral cancer, death	Censored	First date
Lost to follow-up	Censored	Date of last assessment without event
Study ended without distant metastases	Censored	Date of last assessment without event

Overall survival (OS): OS is defined from first administration of neoadjuvant treatment to death any cause.

For patients still alive at the end the study (premature discontinuation or final visit), censoring date will be the last date known alive.

Event	Censoring decision	Event or censored date
Death any cause	Not censored	Date of death
Lost to follow-up	Censored	Date of last contact
Study ended without death any cause	Censored	Date of last visit of follow-up

LRFI, DFS, DDFI and OS will be analysed using Kaplan-Meier method and survival curve will be drawn. Median LRFI, DFS, DDFI and OS survival will be given with their 95% confidence interval. LRFI, DFS, DDFI and OS rates at 3 and 5 years will be given.

Predictive factors of efficacy

Predictive factors of efficacy (see Appendix 11) will be analysed by logistic regression for pathological response and proportional hazard Cox model for DFS including demographics, history of pathology, HER2/RH status, scintigraphy and imaging (PET and mammary DCE-US), pharmacokinetics, immunology, pharmacogenetics, and angiogenesis biomarkers results.

8.2.2 Hypothesis Testing

The primary efficacy endpoint is determined by pathological complete response (pCR) in surgical specimens of mammary tissue and lymph nodes, according to Chevallier's classification (see Appendix 8) and reviewed by an independent Committee (Tumor Histology Independent Review Panel, see Section 10.2).

Statistical hypotheses using single stage phase II Fleming design:

- Type I error, α = 5% one-sided
- Power = 95%

- Lower proportion for rejection of docetaxel + trastuzumab + bevacizumab Arm: p0=15%
- Higher proportion for acceptance of docetaxel + trastuzumab + bevacizumab Arm: p1=35%.

8.2.3 Types of Analyses

8.2.3.1 Efficacy Analysis

Efficacy analysis will be primarily based on intent-to-treat and per-protocol population***s***.

8.2.3.2 Exclusion of Data from Analysis

Safety population

All patients who received at least one dose of study medication will be included in the safety population.

Intent to treat population (ITT)

All patients who have been assigned to a treatment group according to Δ SUV and randomization will be included in the intent to treat population.

Per-protocol population (PP)

All ITT patients with major protocol violations will be excluded from the PP analysis. Protocol violations will be reviewed and validated in the data review meeting by the Study Scientific Committee. Protocol violation included:

- inclusion and non inclusion criteria not met,
- intake of forbidden treatment,
- visit dates not respected,
- missing value for main criterion without premature termination,
- protocol design not respected,
- other protocol violations defined by the Study Scientific Committee.

8.2.3.3 Interim Analysis

A first interim analysis will be conducted on the first half of patients (n = 60) on data before randomization. This analysis will be descriptive and no adjustment for multiplicity will be done for the type I error. The proportions of patients with Δ SUV < 70% and Δ SUV ≥ 70% will be described and the potential differences with the protocol hypotheses (60% and 40%, respectively) will be discussed with the Scientific Committee.

The analysis on the primary endpoint will be conducted after the review of pCR by the independent Committee on all patients. This analysis will also include all the data collected until the surgery.

8.2.4 Safety Data Analysis

All safety parameters will be summarized and presented in tables based on the safety population.

Information on study drug will be summarized by duration, starting dose and dose using descriptive statistics.

Treatment exposure will be summarized as the number of cycles received by each subject, and as the percentage of the planned dose of each agent given at each cycle. The dose-intensity will be described.

Adverse event data will be presented in frequency tables (overall and by intensity) by body system.

For selected events of particular interest [neutropenia, wound healing complications, proteinuria, bleeding/haemorrhage, hypertension, thromboembolic events (venous or arterial), congestive heart failure, gastrointestinal perforation, fistulae, Reversible Posterior Leucoencephalopathy Syndrome (RPLS)] summary tables will be presented for time to first onset of the event and for the total number of episodes. Every occurrence of an event in any subject will be counted in the total number of episodes but successive reports of an identical event in the same phase (treatment, follow-up) will be combined (concatenated) into a one episode if the end date of the earlier event was the same as the start date of the later event, or if the end date of the earlier event was missing.

Laboratory data and vital signs will be presented as summary statistics for each time point using both shift and frequency tables.

All adverse events and abnormal laboratory variables will be assessed according to the CTCAE v4.0 grading system (see Appendix 9).

Cardiac events will be assessed according to NYHA criteria (see Appendix 5).

8.2.5 Other Analyses

Translational studies (pharmacokinetics, immunology, pharmacogenetics, and angiogenesis study) will be conducted in order to establish predictive factors of efficacy. The analyses of these studies are detailed in Appendix 11. Specific statistical analysis plans will be written for each analysis.

8.3 Sample Size

The calculation of the sample size is based on a single stage phase II study using the Fleming design.

A rate of complete response < 15% is considered as insufficient to continue the assement of combination. A rate of complete response ≥ 35% is considered as proof of efficacy of

the combination. Accepting a risk of $\alpha = 5\%$ (type I error, one-sided) and $\beta = 5\%$ (type II error), 48 patients are necessary in the docetaxel + trastuzumab + bevacizumab Arm.

As the docetaxel + trastuzumab arm is the randomized standard Arm, no formal sample size was computed. 24 patients will be included in this Arm to permit a 2:1 randomization ratio.

In all, 72 patients (docetaxel + trastuzumab + bevacizumab: 48 patients; docetaxel + trastuzumab: 24 patients) will allow to evaluate the pCR rates in the two randomized arms.

According to the hypothesis that 60% of patients will have a Δ SUV < 70% before Cycle 2 of neoadjuvant treatment (Berriolo-Riedinger et al. 2008), 48 further patients (with Δ SUV $\geq$ 70%) are necessary (these patients will continue docetaxel + trastuzumab neadjuvant treatment). The total of assessable patients is 120.

Furthermore, approximately 135 subjects will be enrolled in the study taking into account that 10% of the enrolled patients will drop out before randomization, to obtain 72 randomized patients.

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor (source document verification), and the maintenance of a drug–dispensing log by the investigator.

Data for this study will be recorded via an Electronic Data Capture (EDC) system using electronic Case Report Forms. It will be transcribed by the site from the paper source documents onto the eCRF. (In no case is the eCRF to be considered as source data for this trial.) A comprehensive validation check program utilizing front-end checks in the eCRF and back-end checks in the Roche data base will verify the data and discrepancy reports will be generated accordingly and transferred electronically to the eCRF at the site for resolution by the investigator.

10. STUDY COMMITTEES

10.1 Study Scientific Committee

The Scientific Committee will have sole responsibility for the scientific conduct and integrity of the AVATAXHER study.

Responsabilities will include:

- Reviewing and approval of the study documents
- Monitoring of accrual
- Compliance and safety during the conduct of the study
- Development and approval of protocol amendments
- Reviewing of study report, abstracts, posters and publications

10.2 Tumor Histology Independent Review Panel

The Tumor Histology Independent Review Panel will have the responsibility evaluated the pathological response on the operative specimens according to Chevallier's ***and Sataloff's*** criteria.

10.3 HER 2 and Hormonal status Review Committee

The HER 2 and Hormonal status Committee will have the responsibility to review the HER 2 status of breast tumors on the basis of ASCO 2007 criteria at the end of the study.

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PART II: ETHICAL AND ADMINISTRATIVE CONSIDERATIONS

12. ETHICAL ASPECTS

12.1 Good Clinical Practices/Declaration of Helsinki

The trial will be conducted in compliance with the principles of the Declaration of Helsinki, ICH Good Clinical Practices, and the national rules and regulations concerning clinical trials.

12.2 Informed Consent

The investigator, or a doctor assigned by the investigator, is responsible for obtaining informed consent for each patient participating in the trial after clearly explaining the objectives, methods and potential risks of the trial.

The consentment must be given in writing or, if this is impossible, attested by a third party totally independent from the investigator and sponsor. For patients under tutelage, the consentment must be given by their legal guardian. If the patient and legal guardian cannot read, an independent witness must be present during the whole time that the information is given. After the patient and legal guardian have given their oral consent for participation in the study, the witness's signature on the form will attest that the consentment information has been explained and understood.
The investigator must explain that the patient is totally free to accept or refuse to participate in the research and can withdraw his consent at any time for whatever reason without prejudicing the quality of treatment.
Three copies of the information sheet and consent form must be signed and dated by the patient and the investigator/doctor who obtained the patient's consent. One copy is given to the patient, one kept by the investigator and the third one by Roche.
The consentment should be given to Roche in an impregnable sealed envelope designed specifically for this use and provided by the CRA mandated by Roche. The impregnable sealed envelopes must be returned to Roche where they will be archived in a safe to respect confidentiality. After being sealed before leaving the investigational center, the envelopes cannot be opened by anybody except by a Health Authority representative. The sealed envelopes will be destroyed without being opened at the end of the legal archiving period.
If new information comes to light that significantly modifies the potential risks of the study, the consent form will be revised and up-dated if necessary. All patients [including those receiving treatment] must be informed of the new information and give their consent to continue the study.

12.3 Ethics Committee

It is the responsibility of the sponsor to submit the research project to the Ethics Committee (Comité de Protection des Personnes) located in the region where the co-ordinating investigator exercises his activity. Ethics Committee approval and authorization by AFSSAPS must be obtained before the study is started. This approval of

the Ethics Committee must be sent in writing to all the investigators participating in the study.

13. PROTOCOL AMENDMENTS

Major amendments at the sponsor's initiative must be approved by the Ethics Committee and authorised by AFSSAPS before being implemented.
Any modification to the approved protocol cannot be implemented before being approved by the Ethics Committee and authorised by AFSSAPS, except for changes necessary to eliminate an immediate hazard to trial subject or changes involving only logistical or administrative aspects of the study [for example, a CRA change, change of telephone numbers].

14. PREMATURE STUDY TERMINATION

The sponsor reserves the right to terminate the study prematurely at any appropriate moment. Should this be the case, the sponsor and the investigator will ensure that the patient's interests are protected.

15. DOCUMENTATION AND ARCHIVING

15.1 Investigator Study Documents

The investigator must keep adequate and accurate records to document the study progress to allow for later data verification. The documents must be classified into two separate categories: [1] Study investigator file, and [2] patient source documentation.

The investigator file should include the protocol and any modifications, a blank copy of the CRF, the C.P.P. approval and AFSSAPS authorisation, a copy of the informed consent form, the documents for tracking experimental medicines, the documents concerning the investigation team and all other study documents or letters etc.

The patient source documentation should include hospital reports, doctor's/nurse's notes, the patient's diary, laboratory results, ECG, EEG, X-rays, reports of special examinations, the signed consent forms, consultants letters.

The investigators must keep the two categories of documents as described above for at least 15 years after completion a discontinuation of the study

If the investigator wishes to entrust the study files to a third party or move them to another location, Roche must be previously informed.

15.2 Source document verification

The investigator must allow the Clinical Research Assistant to have **direct** access to any or all the study materials needed for source data verification and proper review of the study progress.
During the visit of the CRA, the investigator must provide the CRA with printed copies of the medical file signed by the investigator.
On request, the investigator must provide the sponsor with all necessary records from the study documentation or the patient's medical files. This is especially important when errors in noting data are suspected. In the case of specific problems and/or requests from

the Health Authorities, it is also indispensable to have access to all study data while strictly respecting the confidentiality of patient information.

15.3 Audits and inspections

The investigator agrees to comply with Roche and regulatory authority requirements regarding the auditing of the study or inspection. The verification of data in the CRF must be carried out by comparing it directly with source documents.

15.4 Case Report Form

All the results of assessments performed during the study will be recorded for each patient in a Case Report Form (CRF).
The investigator, or a person designated by the investigator, must complete the CRF at each visit and all document provided by the sponsor (treatment administration record, etc). This also applies to patients that were selected but not included and patients prematurely withdrawn from the study.
Should a patient prematurely withdraw from the study, whatever the reason, a complete final evaluation should be made for the patient and the reason for withdrawal must be recorded in the CRF. In the case of a patient failing to attend an evaluation, the investigator should make every effort to contact the patient and determine the reason for withdrawal and propose an end of study visit. Should a patient withdraw due to an intercurrent disease or adverse event, the investigator should record in the CRF all available and/or appropriate supplementary information.
All persons to whom the investigator designates part of the study follow up must be identified in the center's "Authorization Form".
Any corrections and modifications made to the CRF must be made by the investigator, or person designated by the investigator, according to the instructions given.
At the end of each visit, the investigator must sign and date the CRF to attest the authenticity of the data and its consitency with document source data.
During data validation, the investigator may be requested to make corrections. These requests should be signed and returned as rapidly as possible.
At the end of the study, the investigator will keep a complete copy of the CRFs and correction requests.

15.5. Study Monitoring

The Clinical Research Associate (CRA) mandated by Roche will contact and visit the investigator at regular intervals and will be authorized, on request, to verify the study documents [CRF and other important data].
The CRA is responsible for reviewing the CRFs at regular intervals during the study in order to verify adherence to the protocol and that the information in the CRF is complete, clear and correct. The investigator, or the person designated by the investigator, must cooperate fully with the CRA to ensure that any emergent problems are solved. The CRA is also responsible for monitoring the translational research studies.

15.6. Confidentiality and Subject's Anonymity

The investigator must ensure that the patient's anonymity will be maintained. On all documents submitted to Roche, subjects must be identified only by an identification code, never by their name or hospital/clinical number. The investigator should keep a separate confidential enrolment log which matches an identifying code with the subject's name and address. Documents kept by the investigator that are not for submission to Roche, such as the written consent forms, should be considered as strictly confidential.

15.7. Publication and Proprietary information protection

It is the responsibility of the investigator to publish the clinical study results as soon as the study is completed. In the case of a multicenter trial, the coordinating investigator must ensure that the results of one of the centers are not published before the whole trial results are published.

Roche reserves the right to review the manuscripts before their submission for publication or presentation. This is not intended to restrict or hinder publication or presentation, but to allow Roche to protect proprietary information and to provide comments based on information that may not yet be available to the investigator.

16. APPENDICES

List of appendices

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Appendix 1: ICH GUIDELINES FOR CLINICAL SAFETY DATA MANAGEMENT. DEFINITIONS AND STANDARDS FOR EXPEDITED REPORTING. TOPIC E2 (CONT.)

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event]
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event **are** indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined

follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator file contains the list of persons to be contacted in case of SAE.

Appendix 2: TNM STAGING SYSTEM

TNM Nomenclature

Primary Tumour (T)

T_0	No evidence of primary tumour	
T_{is}	Carcinoma in situ	
T_1	≤2 cm	
	T_{1mic}	≤0.1 cm
	T_{1a}	>0.1 cm but not >0.5 cm
	T_{1b}	>0.5 cm but not >1.0 cm
	T_{1c}	>1.0 cm but not >2.0 cm
T_2	>2 cm but not >5 cm	
T_3	>5 cm	
T_4	Any size, with direct extension to chest wall or skin (only as described below)	
	T_{4a}	Extension to chest wall (excluding pectoral muscle)
	T_{4b}	Oedema (including peau d'orange) or ulceration of skin or presence of satellite skin
		Nodules confined to the same breast
	T_{4c}	Both T_{4a} and T_{4b}
	T_{4d}	Inflammatory carcinoma

Important notes:

Inflammatory carcinoma

Inflammatory carcinoma is a clinicopathologic entity characterized by diffuse erythema and oedema (peau d'orange) of the breast, often without an underlying palpable mass. These clinical findings should involve the majority of the skin of the breast. It is important to remember that inflammatory carcinoma is primarily a clinical diagnosis. Involvement of the dermal lymphatics alone does not indicate inflammatory carcinoma in the absence of clinical findings. In addition to the clinical picture, however, a biopsy is still necessary to demonstrate cancer either within the dermal lymphatics or in breast parenchyma itself.

Skin of Breast

Dimpling of the skin, nipple retraction, or any other skin change, except those described under T_{4b} and T_{4d} may occur in T_1, T_2, or T_3 without changing classification.

Regional lymph nodes (N)

Clinical

NX Regional lymph nodes cannot be assessed (e.g. previously removed)

N_0 No regional lymph node metastasis

N_1 Metastasis to movable ipsilateral axillary lymph node or nodes

N_2 Metastasis to ipsilateral axillary lymph nodes fixed or matted to one another or to other structures; or in clinically apparent* ipsilateral mammary nodes in the absence of clinically evident axillary lymph node metastasis

- N_{2a} Metastasis in ipsilateral axillary lymph nodes fixed to one another (matted) or to other structures
- N_{2b} Metastasis only in clinically apparent ipsilateral mammary nodes and in the absence of clinically evident axillary lymph node metastasis

N_3 Metastasis in ipsilateral infraclavicular lymph node(s), or in clinically apparent* ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement.

- N_{3a} Metastasis in ipsilateral infraclavicular lymph node(s)
- N_{3b} Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
- N_{3c} Metastasis in ipsilateral supraclavicular lymph node(s)

Pathologic(pN)[a]

pNX Regional lymph nodes cannot be assessed (e.g. previously removed, or not removed for pathologic study)

pN0 No regional lymph node metastasis histologically, no additional examination for isolated tumour cells (ITC)

Note: Isolated tumour cells (ITC) are defined as single tumour cells or small cell clusters not greater than 0.2 mm, usually detected only by immunohistochemical (IHC) or molecular methods but which may be verified on H&E stains. ITCs do not usually show evidence of malignant activity, e.g. proliferation or stromal reaction.

- pN0 (i –) No regional lymph node metastasis histologically, negative IHC
- pN0 (i +) No regional lymph node metastasis histologically, positive IHC, no IHC cluster greater than 0.2 mm
- pN0 (mol –) No regional lymph node metastasis histologically, negative molecular findings (RT-PCR)[b]
- pN0 (mol +) No regional lymph node metastasis histologically, positive molecular findings (RT-PCR)[b]

pN_1 Metastasis in 1 to 3 axillary lymph nodes, and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent**

- pN_{1mi} Micrometastasis (greater than 0.2 mm, none greater than 2.0 mm)
- pN_{1a} Metastasis in 1 to 3 axillary lymph nodes

pN_{1b} Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node but not clinically apparent**

pN_{1c} Metastasis in 1 to 3 axillary lymph nodes and in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent.** (If associated with greater than 3 positive axillary lymph nodes, the internal mammary nodes are classified as pN3b to reflect increased tumour burden)

pN_2 Metastasis in 4 to 9 axillary lymph nodes, or in clinically apparent* internal mammary nodes in the absence of axillary lymph node metastasis.

pN_{2a} Metastasis in 4 to 9 axillary lymph nodes (at least one tumour deposit greater than 2.0 mm)

pN_{2b} Metastasis in clinically apparent* internal mammary nodes in the absence of axillary lymph node metastasis

pN_3 Metastasis in 10 or more axillary lymph nodes, or infraclavicular lymph nodes, or in clinically apparent* ipsilateral internal mammary lymph nodes in the presence of 1 or more positive axillary lymph nodes; or in more than 3 axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes

pN_{3a} Metastasis in 10 or more axillary lymph nodes (at least one tumour deposit greater than 2.0 mm), or metastasis to the infraclavicular lymph nodes

pN_{3b} Metastasis in clinically apparent* ipsilateral internal mammary nodes in the presence of 1 or more positive axillary lymph nodes; OR in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent.**

pN_{3c} Metastasis in ipsilateral supraclavicular lymph nodes

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Metastasis (M)

M_0 No distant metastasis

M_1 Distant metastasis, includes separate tumour nodule(s) in a different lobe (ipsilateral or contralateral)

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a Classification is based on axillary lymph node dissection with or without sentinel lymph node dissection.

Classification based solely on sentinel lymph node dissection without subsequent axillary lymph node dissection is designated (sn) for "sentinel node" e.g. pN0(i+)(sn)

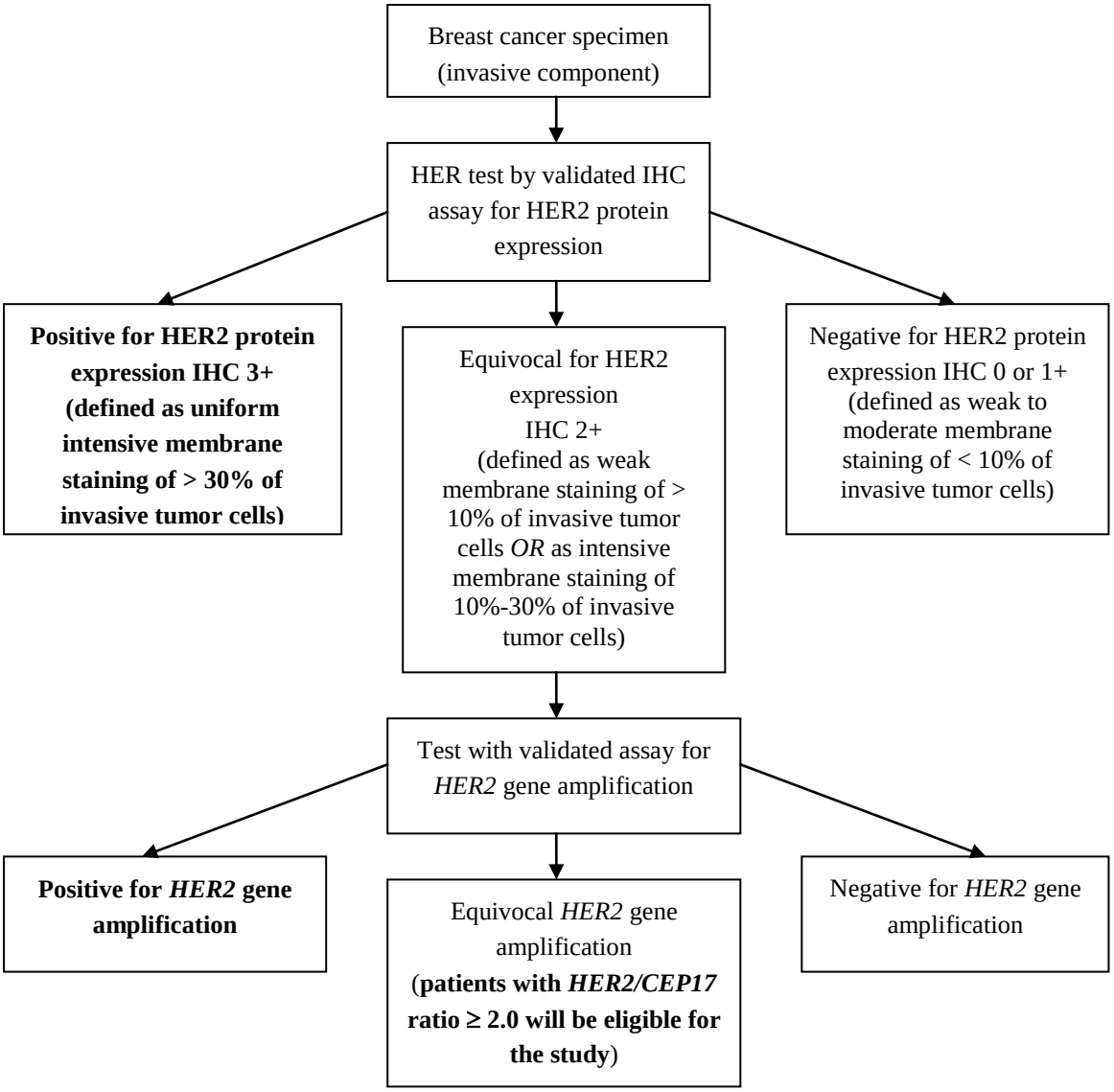
b RT-PCR: reverse transcriptase/polymerase chain reaction

* Clinically apparent is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination.

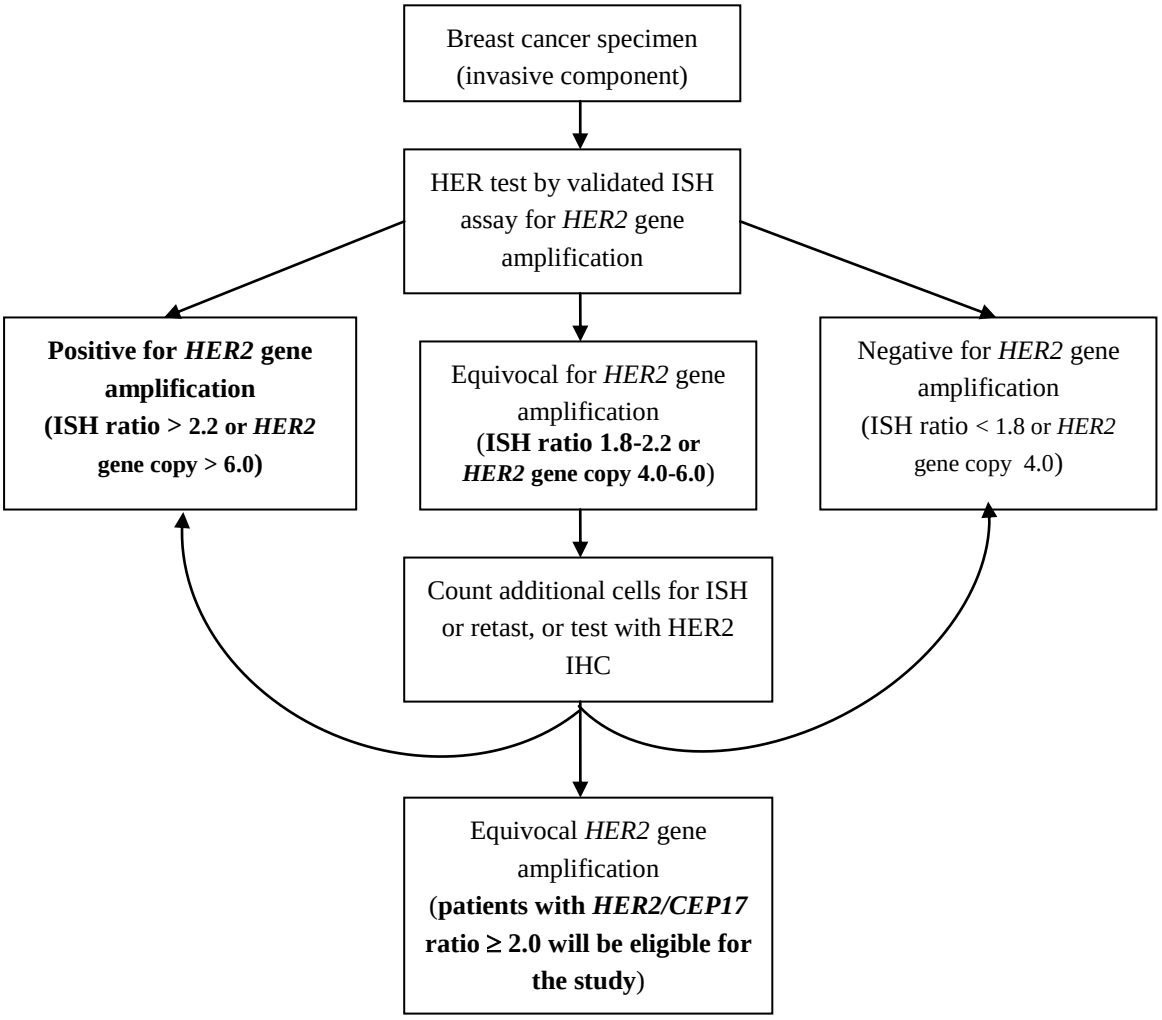
** Not clinically apparent is defined as not detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination.

Appendix 3: GUIDELINES FOR HER2 TESTING IN BREAST CANCER

1. IHC algorythm



2. ISH algorythm



American Society of Clinical Oncology/College of American Pathologists. Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. J Clin Oncol 2007;25(1):118-45.

Appendix 4: ECOG PERFORMANCE STATUS SCALE

ECOG	
0	Fully active; able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory
2	Ambulatory and capable of self-care; but unable to carry out any work activities.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled

Appendix 5: NYHA CLASSIFICATION

Clinical Evaluation of Functional Capacity of Patients
with Heart Disease in Relation to Ordinary Physical Activity

NYHA	Functional Class	Description	Objective Assessment
I	Mild	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation or dyspnoea.	No objective evidence of cardiovascular disease.
II	Mild	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnoea	Objective evidence of minimal cardiovascular disease
III	Moderate	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation or dyspnoea.	Objective evidence of moderately severe cardiovascular disease.
IV	Severe	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

The Criteria Committee of the New York Heart Association. *Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels*. 9th ed. Boston, Mass: Little, Brown & Co; 1994:253-256

Appendix 6: [18F]-FDG-PET PROCEDURES

Les patientes seront recrutées dans chaque centre investigateur par les services d'oncologie, de chirurgie ou gynécologie après indication d'une chimiothérapie néoadjuvante avant chirurgie par la réunion de concertation pluridisciplinaire.

Les examens seront réalisés dans les services de Médecine Nucléaire de différents sites selon un protocole commun.

Une TEP préthérapeutique (TEP1) sera réalisée au moins 10 jours après la biopsie et le plus près possible de tout traitement (7 jours). Elle comportera 2 acquisitions, la première centrée sur la région mammaire, la deuxième sera un Corps Entier (CE) standard.

La deuxième TEP (TEP2) sera réalisé immédiatement avant la deuxième cure de chimiothérapie néoadjuvante, avant tout traitement avec des corticoïdes. Seule l'acquisition centrée sur la région mammaire sera réalisée.

Réalisation de l'examen TEP [^{18}F]-FDG

1) PREPARATION DES PATIENTES

Absence d'effort musculaire / sportif important la veille de l'examen

Patiente à jeun depuis 6 heures

- pas d'alimentation solide ou liquide pouvant contenir du glucose ou d'autres sucres
- pas de tabac

Hydratation abondante (eau plate ou gazeuse non édulcorée et non sucrée)

Pas de gomme à mâcher

Miction avant installation

Un cathéter veineux (Bras controlatéral ou membre inférieur) sera posé avec une perfusion de 500 ml de sérum physiologique (pas d'utilisation d'une éventuelle voie centrale existante)

Mesure de la glycémie :

Glycémie capillaire : elle permettra rapidement d'exclure une patiente présentant une hyperglycémie > 11 mmol/L)

Glycémie plasmatique (prélèvement périphérique) : elle sera utilisée pour la correction de la SUV. Elle sera prélevée immédiatement avant l'injection de [^{18}F]-FDG.

En l'absence de mesure de la glycémie plasmatique, la glycémie capillaire sera utilisée pour la correction de la SUV.

2) PROTOCOLE

Selon la machine TEP utilisée, une ***dose de 2 à 5 MBq/kg de [^{18}F]-FDG*** sera administrée par injection intraveineuse.

La patiente restera au repos (décubitus dorsal idéalement) pendant la ***phase de captation : 60 à 80 minutes***. Elle devra être au calme et au chaud afin d'éviter la fixation de [^{18}F]-FDG dans la graisse brune.

Le poids, la taille, la glycémie, la dose administrée, le site d'injection, le délai entre l'injection et l'acquisition des images seront notés sur la fiche TEP.

Après avoir vidé la vessie, la patiente sera positionnée dans la machine TEP.

TEP 1

a) Acquisition mammaire : Elle débutera impérativement à 90 minutes post injection du [^{18}F]-FDG. Elle sera centrée sur les seins et les creux axillaires en procubitus, les bras au-dessus de la tête si possible. Un matelas dédié (mammoscintigraphie ou similaire est recommandé). La durée prévisible de cette acquisition est d'environ 10 minutes, elle comportera 2 pas minimum de 4 minutes chacun.

b) Acquisition Corps Entier (mi tête→genoux) : Elle sera réalisée en première intention ou après l'acquisition mammaire définie en a), selon la caméra TEP/TDM [6] et à condition de respecter le délai impératif de 90 minutes post injection pour l'acquisition mammaire. Elle sera réalisée en décubitus dorsal, les bras au dessus de la tête sauf si la position ne peut pas être tolérée. La durée de chaque pas devra être adaptée à l'activité administrée et en aucun cas elle ne doit être inférieure à 2 minutes par pas.

La durée totale prévisible de l'ensemble des acquisitions est d'environ 35 minutes.

TEP 2

Reprendre le poids de la patiente, celui-ci ayant pu changer.

La même activité/kg sera injectée.

Le délai de captation sera identique à celui de la TEP1, c'est à dire l'acquisition mammaire devra impérativement débuter à 90 minutes après l'injection.

Acquisition mammaire centrée sur les seins et les creux axillaires en procubitus, les bras au-dessus de la tête si possible. La durée totale sera d'environ 10 minutes (au moins 2 pas de 4 minutes chacun).

[6] Certaines caméras rapides permettent l'acquisition « corps entier » en moins de 20 minutes, permettant de réaliser l'acquisition mammaire en seconde intention sans risque de dépasser les 90 minutes requises. Pour les autres, il sera recommandé de débuter par l'acquisition mammaire pour respecter le délai requis.

Pas d'acquisition Corps Entier (CE) requise pour le protocole, chaque centre restant libre de la réaliser selon les pratiques locales.

Tous les examens comporteront des acquisitions d'émission et de transmission.

Calibration SUV des caméras :

Chaque centre participant devra fournir les documents prouvant la réalisation au cours des 3 derniers mois d'un calibrage SUV de la machine selon les normes NEMA (National Electric Manufacturers Association) et les recommandations de l'industriel fabricant.

Reconstruction des données :

Les données seront reconstruites à l'aide d'un algorithme itératif propre à chaque type de machine, permettant d'obtenir des images dans les trois axes de l'espace selon les plans coronal, sagittal et transaxial et des images MIP (Maximum Intensity Projection) en mode ciné.

Sauvegarde des données

Les données reconstruites seront sauvegardées au format DICOM EXPORT et enregistrées sur CD-ROM (ces données seront anonymisées en cas de sortie du centre..

Interprétation des images

Les examens seront interprétés dans chaque centre avant le 3ème cycle.

Si la patiente est métastatique (atteinte extra ganglionnaire et/ou ganglionnaire médiastinale) ou si la captation de [^{18}F]-FDG est faible (défini par un SUV ≤ au SUV du parenchyme mammaire controlatéral), elle sera exclue de protocole.

Si une atteinte ganglionnaire mammaire interne ou sus-claviculaire est détectée, la patiente ne sera pas exclue du protocole.

La mesure de la captation tumorale de [^{18}F]-FDG et des éventuelles métastases ganglionnaires sera estimée pour chaque examen par la mesure de la SUV maximale dans le volume tumoral corrigée par le poids (SUV_{max}). Les SUV de la tumeur et des ganglions seront normalisées par la Surface Corporelle (SC) et la glycémie (G) :

$$SUV_{max\text{-}SC\text{-}G} = 70 \times SUV_{max} \times SC \times G / (P \times 1{,}72 \times 5{,}6)$$

SC en m^2 : 0.007184×TBW0.425×H0.725

G en mmol/L ; P en kg

La variation de la SUV de la tumeur ΔSUV-t (%) ou des ganglions ΔSUV-gg (%) sera calculée selon les formules suivantes :

$\Delta SUV\text{-}t\ (\%) = 100 \times (SUV_{tep1} - SUV_{tep2}) / SUV_{tep1}$.

$\Delta SUV\text{-}gg\ (\%) = 100 \times (SUV_{tep1} - SUV_{tep2}) / SUV_{tep1}$.

Le seuil retenu pour la randomisation des patientes est : $\Delta SUV\text{-}t \geq 70\%$

1. Kim CK, Gupta NC, Chandramouli B, Alavi A. Standardized uptake values of FDG: body surface area correction is preferable to body weight correction. J Nucl Med 1994;35(1):164-7
2. Lindholm P, Minn H, Leskinen-Kallio S, Bergman J, Ruotsalainen U, Joensuu H. Influence of the blood glucose concentration on FDG uptake in cancer-a PET study. J Nucl Med. 1993 Jan;34(1):1-6.
3. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition. 1989 Sep-Oct;5(5):303-11; discussion 312-3.

Appendix 7: PROTEINURIA ALGORITHM FOR BEVACIZUMAB-RELATED PROTEINURIA

Proteinuria will be assessed within 48 hours before each bevacizumab treatment by dipstick method unless assessed by 24-hour urine collection.
Algorithms for the appropriate assessment following a positive dipstick result with corresponding bevacizumab treatment management guidance are provided in following Figures. If bevacizumab treatment is delayed for more than 2 cycles, continuation of treatment must be discussed with the Roche medical monitor or designee.

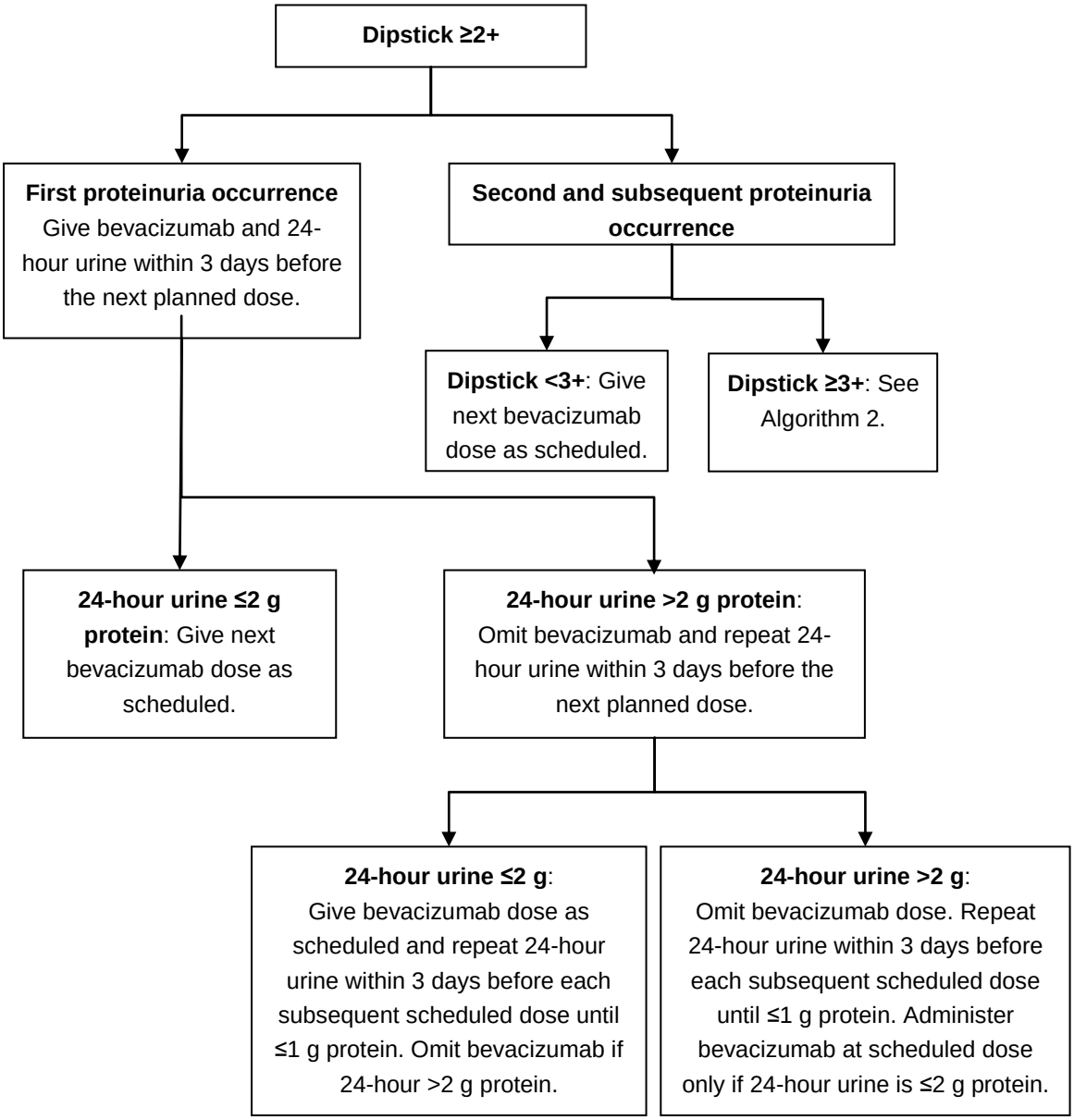
First occurrence of proteinuria during treatment with bevacizumab (algorithm 1 below)

- **< 2+** proteinuria (dipstick): administer bevacizumab as planned

- **≥ 2+** proteinuria (dipstick): administer bevacizumab as planned and collect 24-hour urine for determination of total protein within 3 days before the next scheduled bevacizumab administration:
  - **If 24-hour proteinuria ≤ 2 g**: administer next bevacizumab dose as scheduled
  - **If 24-hour proteinuria > 2 g**: omit next scheduled bevacizumab dose and do 24-hour urine collection for determination of total protein within 3 days before the subsequently scheduled cycle. Delay bevacizumab treatment until proteinuria has decreased to ≤2 g. Do 24-hour urine before each scheduled dose until proteinuria has improved to ≤1 g/24 hours, but omit bevacizumab only if > 2 g/24 hours

- **Nephrotic** syndrome (Grade 4, NCI CTC-AE v4.0): Permanently discontinue bevacizumab treatment.

Algorithm 1 for proteinuria: first occurence



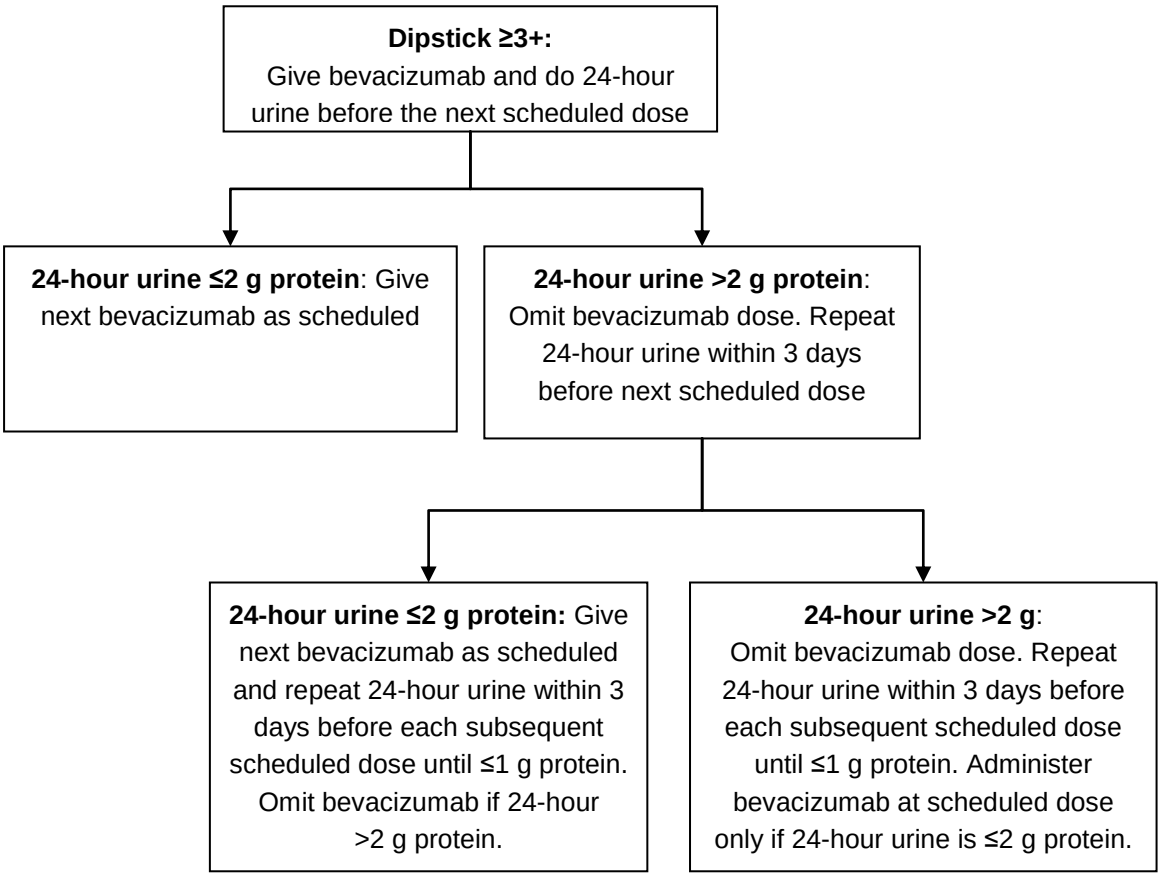
Second and subsequent occurrence of proteinuria during treatment with bevacizumab (algorithm 2 below)

- < **3+** proteinuria (dipstick): administer bevacizumab as planned
- ≥ **3+** proteinuria (dipstick): administer bevacizumab as planned and collect 24-hour urine for determination of total protein within 3 days before the next scheduled bevacizumab administration:

- **If 24-hour proteinuria ≤ 2 g**: administer next bevacizumab dose as scheduled
- **If 24-hour proteinuria > 2 g**: omit next scheduled bevacizumab dose and do 24-hour urine collection for determination of total protein within 3 days before the subsequently scheduled cycle. Delay bevacizumab treatment until proteinuria has decreased to ≤2 g. Do 24-hour urine before each scheduled dose until proteinuria has improved to ≤1 g/24 hours, but omit bevacizumab only if > 2 g/24 hours

▪ Nephrotic syndrome (Grade 4, NCI CTC-AE v4.0): Permanently discontinue bevacizumab treatment

Algorithm 2 for proteinuria: second and subsequent occurrences



Appendix 8: CHEVALLIER'S AND SATALOFF'S CRITERIA OF PATHOLOGICAL RESPONSE

The pathological complete response will be assessed in surgical specimens of mammary tissue and lymph nodes, according to:

- Chevallier's classification (***local procedures*** and review by an independent Committee).

Grade 1:	**Grade 2:**	**Grade 3:**	**Grade 4:**
Disappearance of All Tumors Either in the Breast or in the Nodes	Persistence of Carcinoma In Situ in the Breast Only. No Nodal Invasion	Presence of Invasive Carcinoma With Stromal Alteration	Presence of Invasive Carcinoma Without Modification

pCR is defined as the absence of invasive cancer cells in both the primary site and lymph nodes (grade 1 and 2).

- Sataloff's classification (review by an independent Committee).

The pathological complete response will be assessed in surgical specimens of mammary tissue and lymph nodes, according to Sataloff's classification and reviewed by an independent Committee.

Tumor (T)

TA:	**TB:**	**TC:**	**TD:**
Total or near total therapeutic effect	**Subjectively >50% therapeutic effect but less than total or near total**	**<50% therapeutic effect but effect evident**	**No response**

Node (N)

NA:	**NB:**	**NC:**	**ND:**
No nodal metastasis and evidence of therapeutic effect	**No nodal metastasis or therapeutic effect**	**Nodal metastasis present with evidence of therapeutic effect**	**Nodal metastasis present without evidence of therapeutic effect**

pCR is defined as tumor A (TA)/node A (NA) or TA/NB.

Appendix 9: NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

The NCI CTC-AE version 4.0 is located on the internet at the following URL:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev4.pdf

Appendix 10: TUMOUR ASSESSMENTS (MODIFIED RECIST CRITERIA)

Presented here is the official quick reference guide provided by the National Cancer Institute.

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter $\geq$ 20 mm using conventional techniques or $\geq$10 mm with spiral CT scan.

Non-measurable lesions - all other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques;

All measurements should be taken and recorded in metric notation, using a ruler or callipers. All screening evaluations should be performed as closely as possible to the beginning of treatment and never more than 28 days before treatment start.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at screening and during follow up.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by colour photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement

CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumours of the chest, abdomen and pelvis. Head and neck tumours and those of extremities usually require specific protocols.

Lesions on chest X-ray are not acceptable as measurable lesions. Instead, CT (or MRI) is required.

When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumour lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

The utilisation of endoscopy and laparoscopy for objective tumour evaluation has not yet been fully and widely validated and must not be used. Tumour markers must not be used to assess response.

Histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumour types such as germ cell tumours).

Screening documentation of "Target" and "Non-Target" lesions

All measurable lesions up to a maximum of five lesions per organ and **10** lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at screening.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for ***all target lesions*** will be calculated and reported as the screening sum LD. The screening sum LD will be used as reference by which to characterize the objective tumour.

All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at screening. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow up.

Response Criteria

	Evaluation of target lesions
Complete Response (CR)	Disappearance of all target lesions
Partial Response (PR)	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the screening sum LD
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started
	Evaluation of non-target lesions
Complete Response (CR)	Disappearance of all non-target lesions and normalisation of tumour marker level
Incomplete Response/Stable Disease (SD)	Persistence of one or more non-target lesion(s) or/and maintenance of tumour marker level above the normal limits
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions [A]

[A] Although a clear progression of "non target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of best overall response

The best overall response is the best response recorded from treatment start until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, investigation of the residual lesion can be considered (biopsy) to confirm the complete response status.

Confirmation

The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

To be assigned a status of PR or CR, changes in tumour measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

In the case of SD, follow up measurements must have met the SD criteria at least once after study entry at a minimum interval, not less than 6 weeks.

Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of stable disease

SD is measured from the start of the treatment, until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

Appendix 11: TRANSLATIONAL RESEARCH STUDIES

Introduction

Several **predictive factors studies** will be conducted alongside the main protocol at all participating sites. They are optional (facultative). Their objective is to **identify predictive factors of efficacy and safety.**

These studies will investigate the following areas:

- pharmacokinetics and pharmacokinetic-pharmacodynamic (study n° 1)
- immunology (study n°2)
- pharmacogenetics (study n° 3)
- angiogenesis study (study 4): angiogenesis biology (study n° 4a) and mammary DCE-US (study n° 4b)

For these studies, **blood samples** will be collected and **extra imaging** will be performed.

For these studies, specific examinations/samplings are described in details in the informed consent form (one specific consent for each study). However, as the **angiogenesis study** (study n° 4a) will be conducted only in the investigating center of Dijon, a **specific information form** with an additional paragraph detailing the angiogenesis study will be edited and provided to this center.

Figure 5: Schedule of biologic Assessments for Predictive Factors Studies

	Study	**PK and PK-PD ***	**Immunology**	**Pharmaco -genetic**	**Angiogenesis** *(only for Dijon centre)*
Neoadjuvant cycles	Nature of samples	blood	blood	blood	blood
Cycle 1	Before trastuzumab infusion	**x**[a]	**x**[b]	**x**[c]	**x**[d]
	2h after end of trastuzumab infusion	**x**[a]			
Cycle 2	Before docetaxel infusion	**x**[a]			**x**[d]
	2h after end of docetaxel infusion	**x**[a]			
Cycle 3	Before trastuzumab and bevacizumab infusions	**x**[a]			**x**[d]
	2h after end of trastuzumab and bevacizumab infusions	**x**[a]			
Cycle 4	Before trastuzumab and bevacizumab infusions	**x**[a]			**x**[d]
	2h after end of trastuzumab and bevacizumab infusions	**x**[a]			
Cycle 5	Before trastuzumab and bevacizumab infusions	**x**[a]			**x**[d]
	2h after end of trastuzumab and bevacizumab infusions	**x**[a]			
Cycle 6	Before trastuzumab and bevacizumab infusions	**x**[a]			**x**[d]
	2h after end of trastuzumab and bevacizumab infusions	**x**[a]			

x[a]: 1 sample of 5 ml (2 x 5 ml for each cycle) **x**[c]: 1 sample of 5 ml

x[b]: 2 samples of 5 ml **x**[d]: 1 sample of 25 ml

* If a Cycle is delayed, a 3rd blood sample will be necessary, just 21 days after the previous Cycle.

Study n° 1: Pharmacokinetic (PK) and pharmacokinetic-pharmacodynamic (PK-PD) Study

This exploratory study is coordinated by **Pr Gilles Paintaud**, UMR CNRS 6239 GICC, Pharmacology of Therapeutic Antibodies, Tours University, France.

Objectives

- To describe patient exposure by measuring bevacizumab and trastuzumab concentrations during the neoadjuvant treatment.
- To study if pathological complete response rate, DFS and OS are influenced by bevacizumab and trastuzumab serum concentrations.
- To study if patients developing certain adverse events during bevacizumab or trastuzumab treatment have higher serum concentrations than those who do not develop these adverse events.

Endpoints

- trough bevacizumab and trastuzumab concentrations, obtained before each infusion and 2 hours after the end of the infusion (peak) during neoadjuvant period. When bevacizumab and trastuzumab are administered on the same day, a single "peak" concentration will be collected, after the second infusion.
- individual pharmacokinetic parameters of bevacizumab and trastuzumab, estimated by compartment PK modelling.
- pCR, DFS, OS.
- individual pharmacodynamic parameters describing the concentration - effect of bevacizumab and trastuzumab, estimated by PK-PD modelling.
- adverse events of specific interest (hypertension and proteinuria)

Rationale

As shown for all therapeutic monoclonal antibodies (Ternant 2005), there is an interindividual pharmacokinetic variability of bevacizumab and trastuzumab.

Bevacizumab

Although a proportional increase in bevacizumab concentrations with dose was shown (Gordon 2001), patient exposure as measured by area under the concentration versus time curves (AUC) has an interindividual variability of 53% and 39% after 3 mg/kg and 10 mg/kg, respectively (Gordon 2001). A population pharmacokinetic study using a two-compartment model estimated the interindividual variability of clearance and central volume of distribution to be 44% and 25%, respectively (Hsei 2001). A phase I/II study reported interindividual variability of clearance and central volume of distribution of 31% and 21%, respectively, with a resulting 40% variability of AUC (Herbst 2005).

Patients with low albumin plasma concentrations have a higher clearance of bevacizumab than patients with normal albumin concentrations (Gaudreault 2001, Kuebler 2005). Bevacizumab pharmacokinetics are also influenced by weight, sex and tumour volume (Kuebler 2005).

This interindividual pharmacokinetic variability is relevant since bevacizumab concentrations were found to influence clinical response in lung cancer (study #4, Avastin FDA application): patients with an AUC > 4050 µg/mL.day had a median time to progression of 293 days as compared with 84 days for patients with an AUC ≤ 4050 µg/mL.day. Conversely, bevacizumab clearance and volume of distribution had a significant influence on median time to progression.

The analysis of clinical data on toxicity suggests that arterial hypertension and proteinuria occurring during bevacizumab treatment are dose-dependant. However, no data is available on the relationship between bevacizumab serum concentrations and adverse drug reactions. If such a relationship exists, it would help to identify, in the early phase of their treatment, the patients who are at risk of developing certain side effects.

Trastuzumab
An interindividual pharmacokinetic variability was also shown for trastuzumab (Bruno 2005, Baselga 2005)., Trough concentrations measured 7 and 8 weeks after treatment initiation were higher in complete responders (70.3 µg/mL) and partial responders (58.4 µg/mL) than in non-responders (44.3 µg/mL, $p < 0.001$) (Baselga 2001). The measurement of trastuzumab concentration is serum samples may therefore explain part of the differences between patients that are going to be observed in this study.

Samples calendar

For each patient:

- 2 supplementary blood samples (2 x 5 mL) will be drawn on 6 times during the neoadjuvant treatment period (C1 to C6). If a Cycle is delayed (more than 21 days after the previous Cycle), a 3rd blood sample will be necessary, just 21 days after the previous Cycle.

For this study, a specific informed consent item will be signed by patients in the consent form before the blood sample.

Centralization and Processing of samples

Blood samples will be sent to Biological Resource Centre of Touraine (CBR-T, Certified Iso 9001 : 2000) and measured by UMR CNRS 6239 using validated ELISA techniques (Ternant, manuscript; Darrouzain, manuscript).
Samples will be stored for 5 years.

PK and PK-PD modelling

Using population PK modelling, the serum concentrations of the two antibodies will be used to estimate individual pharmacokinetic parameters and exposure (AUC). Using clinical and biological endpoints together with serum concentrations, individual PK-PD

parameters will be estimated. The influence of pharmacogenetic factors (*FCGR3A, FCGR2A* and *VEGF* polymorphisms) on the PK-PD parameters will be analysed.

The concentrations of the therapeutic antibodies and the estimated PK and PK-PD parameters will be incorporated into the final database.

References

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Study n° 2: Immunology Study

This exploratory study is coordinated by **Pr Gilles Thibault**, UMR CNRS 6239, laboratory of Immunology, Tours University, France.

Objectives

- to study whether the pCR,DFS and OS are influenced by the number of peripheral blood CD56+CD16+ NK cells before therapy.
- to study whether the pCR, DFS and OS are influenced by the number of peripheral blood CD16 positive cells before therapy.
- to study whether the number of peripheral blood CD16 positive cells is related to the results of ΔSUV, studied with PET.

Endpoints

- Enumeration of CD3+ T cells, CD19+ B cells, and CD3-CD56+CD16+ NKcells,
- Enumeration of CD3-CD56++CD16+ NKcells, CD3-CD56-CD16+ NK cells, TCRαβCD16+ T cells, TCRγδ+CD16+ T cells and CD14+CD16+ myeloid cells.
- pCR, DFS, RFI, OS.
- ΔSUV

Rationale

Several studies suggest that antibody-dependent cellular cytotoxicity (ADCC) is involved in the mechanism of action of trastuzumab. In FcR-γ knock-out nude mice, trastuzumab was unable to control an Her-2 psitive tumor xenograft, conversely to control nude mice [Clynes et al.]. FcR-γ is an immune tyrosine-based activation motif (ITAM)-containing chain, required for the expression and signalling of "activatory" FcγRs, *i.e.* Fc γ RI, FcγRIII and also FcγRIV in mice. Similarly the F(ab')2 fragment of trastuzumab does not inhibit the growth of the JIMT-1 in SCID mice conversely to the compelte IgG1 (Barok et al.). In a pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2, patients with complete remission or partial remission were found to have a higher capability to mediate *in vitro* ADCC and a higher *in situ* infiltration of leukocytes (Gennari et al.). It was then demonstrated that trastuzumab treatment was associated with significantly increased numbers of tumour-associated NK cells compared with controls (Arnould et al.). More recently the clinical response to trastuzumab has been shown to be influenced by the V158F polymorphism of the *FCGR3A* gene encoding the FcγRIIIA receptor involved in ADCC. The influence of the H131R polymorphism of *FCGR2A* has also been reported (Musolino et al.), although it may be related to its linkage disequilibrium with the V158F polymorphism of the *FCGR3A* (Lejeune et al.). This result demonstrate that the therapeutic effect of trastuzumab is dependent on CD16 positive cells, which include the majority of NK cells, a fraction of TCRαβ+ (Clemenceau et al.) and TCRγδ+ T cell and a fraction of monocytes/macrophages. Moreover, the latter, which is known to affect the affinity of monoclonal antibodies for the receptors (Dall'Ozzo et al.), influences trastuzumab-mediated ADCC against Her-2 positive cells exerted by lymphocytes from breast cancer patients (Musolino et al.). Finally, the *in vitro* trastuzumab-mediated ADCC

of patient's PBMCs (peripheral blood mononuclear cells) before therapy, which was significantly dependent on the quantity of CD16+CD56+ lymphocytes among PBMC (Varchetta et al.), has been found to be associated or correlated with clinical response to trastuzumab (Varchetta et al., Musolino et al. Beano et al.). Theses studies indicate that the number of CD16 positive cells should be evaluated in clinical studies analysing the efficacy of trastuzumab.

Sample calendar
For each patient, 2 supplementary blood samples (2 x 5 mL, EDTA) will be withdrawn before neoadjuvant trastuzumab treatment.
For this study, a specific informed consent item will be signed by patients in the consent form before the blood sample.

Centralization and Processing of samples
Blood sample will be sent to local immunology laboratories for enumeration of CD3+ T cells, CD19+ B cells, and CD3-CD56+CD16+ NKcells.
Blood sample will be sent within 24 h to the laboratory of Immunology of Tours (Pr. G. Thibault, Pr. H. Watier) for the enumeration of CD3-CD56++CD16+ NKcells, CD3-CD56-CD16+ NK cells, TCRαβCD16+ T cells, TCRγδ+CD16+ T cells and CD14+CD16+ myeloid cells.
Samples will be stored for 5 years.

Statistical analysis
Data collected for this immunology study will be incorporated into the final database.

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Study n° 3: Pharmacogenetic Study

This exploratory study is coordinated by: **Pr Gilles Paintaud**, UMR CNRS 6239 GICC, Pharmacology of Therapeutic Antibodies, Tours University, France and Pr Gilles Thibault

Objectives

- To describe *FCGR3A, FCGR2A* and *VEGF* genetic polymorphisms distribution in the study population
- To study if pCR, DFS and OS are influenced by *FCGR3A, FCGR2A* or *VEGF* genetic polymorphisms
- To study if patients developing certain adverse events during bevacizumab and trastuzumab treatment have specific *FCGR3A, FCGR2A* or *VEGF* genotypes.

Endpoints

- *FCGR3A* and *FCGR2A* genetic polymorphism
- *VEGF* genetic polymorphism
- pCR, DFS, OS
- adverse events

Rationale

- **FCGR polymorphisms**

Several studies (Tokuda 1996, Gennari 2004, Mimura 2005) have shown that trastuzumab acts by antibody-dependent cellular cytotoxicity (ADCC). Since the genetic polymorphism of *FCGR3A*, the gene coding FcγRIIIa, a receptor of the Fc portion of immunoglobulins, influences the efficacy of monoclonal antibodies acting by ADCC (Watier 2005), it is expected to influence the efficacy of trastuzumab in treated patients.

This was recently confirmed by the work of Musolino (2008). Fifty-four consecutive patients with HER-2/neu-amplified breast cancer receiving trastuzumab plus taxane for metastatic disease were analysed. The *FCGR3A* genotype significantly influenced objective response rate and progression-free survival.

Because of the magnitude of this effect, all clinical studies analysing the efficacy of trastuzumab should include *FCGR3A* genotyping, in order to interpret the differences in response between patients. The role of *FCGR2A-131* polymorphism is also discussed (Musolino 2008). Although it may be related to its genetic disequilibrium with *FCGR3A*-158 polymorphism (Ternant 2005), it is necessary to analyse this genotype as well.

- **VEGF polymorphisms**

Several polymorphisms in the VEGF gene have been reported and may influence VEGF impact in solid tumors.

Schneider et al. (2008) have recently shown an association of VEGF genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel + bevacizumab in advanced breast cancer. Using our nomenclature, the four polymorphisms described by this author are: -2549, -1154, +405 and +936. We have identified a 5th polymorphism (-460) whose analysis will also be included in this study.

Genetic analyses will be performed at the end of the inclusion of the patients.

Sample calendar
For each patient, 1 supplementary blood sample (1 x 5 mL) will be withdrawn at any time of the neoadjuvant treatement period.
For this study, a specific informed consent item will be signed by patients in the consent form before the blood sample.

Centralization and Processing of samples
Blood sample will be sent to Centre de Ressources Biologiques de Touraine (CRB-T) where DNA will be extracted and stored until analysis. Genotyping will be performed in the laboratory of Immunology of Tours (Pr. G. Thibault, Pr. H. Watier). Transfer of the samples for CRB-T to the department of immunology and transmission of the results to the investigators will be coordinated by the team of to Pr Gilles Paintaud.
Samples will be stored for 5 years.

Statistical analysis
Results of this pharmacogenetic study will be incorporated into the final database.

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Study n° 4: Angiogenesis Biomarkers Study

This exploratory study is coordinated by: **Dr Bruno Coudert**, GF Leclerc Centre, Medical Oncology department, Dijon, France.

Study 4a: Angiogenesis Biology

Objectives

- To describe the variation of CEC (Circulating Endothelial Cells), VEGF, and its soluble receptor VEGFR2, and endothelial and adhesion cell markers, like E-Selectin, P-Selectin, or ICAM-1 levels between screening period (before first administration of neoadjuvant treatment), and before each subsequent neoadjuvant cycle until cycle 6, whatever the treatment arm.
- To evaluate the predictive value of the variation of CEC, VEGF, and its soluble receptor VEGFR2, and endothelial and adhesion cell markers, like E-Selectin, P-Selectin, or ICAM-1 levels on disease free survival (DFS) and overall survival (OS) after a neoadjuvant treatment with bevacizumab, docetaxel and trastuzumab
- To correlate CEC, VEGF, and its soluble receptor VEGFR2, and endothelial and adhesion cell markers, like E-Selectin, P-Selectin, or ICAM-1 levels with pathological response

Endpoints

- CEC, VEGF, and its soluble receptor VEGFR2, and endothelial and adhesion cell markers, like E-Selectin, P-Selectin, or ICAM-1 levels before starting neoadjuvant treatment and during treatment, before each subsequent neoadjuvant cycle until cycle 6, whatever the treatment arm, variation of levels between these evaluations
- DFS and OS at 3 and 5 years,
- Pathological response

Rationale

Quantification of tumor angiogenesis and the measurement of antiangiogenic drug activities in patients remain unresolved issues. Many approaches have been tested in pre clinical and clinical studies, but to date, none has been validated for routine use in patients.
For example, there is currently no defined marker that predicts for clinical benefit to bevacizumab, akin to HER2-neu over expression predicting response to trastuzumab (Seidman et al. 2001). However, many potential surrogate markers are under investigation for the early prediction of drug activity, response to therapy, rapid identification of resistance to treatment, and even clinical outcome. The complexity of tumor angiogenesis, and the multiple regulatory mechanisms of adaptation to angiogenesis during therapy suggests in fact, that multiple and different biomarkers will

be necessary to obtain a comprehensive representation of angiogenesis therapeutic modulation.

Circulating angiogenic factors and related molecules:

Circulating angiogenic factors (pre-treatment blood levels, and their variations during the antiangiogenic treatment), such as soluble VEGF, and its soluble receptor VEGFR2, but also endothelial and adhesion cell markers, like E-Selectin, P-Selectin, or ICAM-1, could also reflect the anti angiogenic activity. Rising levels of circulating VEGF were observed in response to antiangiogenic drugs, possibly reflecting tumor-induced drug hypoxia (Bocci et al. 2004). In a recent phase II clinical trial, breast cancer patients treated with bevacizumab in combination with docetaxel, higher pre-treatment E-Selectin and ICAM-1, and a greater decline of these marker after the first treatment cycle, were significantly associated with response (Ramaswamy et al. 2006). Despite these encouraging results, no validated circulating surrogate biomarkers of antiangiogenesis is currently available for routine clinical use.

Circulating cells:

Circulating endothelial cells (CEC) are, as well as circulating bone marrow-derived endothelial progenitor cells (CEP) involved in the tumor vascularization process, and thereby proposed as quantitative pharmacodynamic surrogate markers for antiangiogenic drug activity (Shaked et al. 2005a). These cells can be easily monitored with a four color flow cytometric assay on whole blood sample (Jacques et al. 2008). Increased numbers of CEC and CEP are observed in the blood of patients with cancer (Bertolini et al. 2006), and their level return to normal following antiangiogenic treatment in preclinical mice models (Shaked et al. 2005b). In a recent clinical study, their kinetics and viability seems to predict survival in breast cancer patients receiving metronomic antiangiogenic chemotherapy (Mancuso et al. 2006). Furthermore, Bevacizumab seems to reduce the frequency of viable CEC and CEP in patients with rectal cancer (Willett et al. 2005), suggesting their potential value as surrogate markers of drug efficacy.

The possibility of measuring molecular, biological or functional parameters of tumor angiogenesis, and monitoring their changes in patients during tumor progression or antiangiogenic therapy remains a major challenge in translational cancer research, both for the clinical validation of mechanisms originally described in experimental models, and the generation of novel mechanistic hypotheses. Such biomarkers may allow the design of customized therapies that maximize benefits and minimize costs. Thus, surrogate markers of angiogenesis contribute to “bench-to-bedside” and “back to bench” translational research.

Samples calendar

This study will only be hold in the investigator center of Dijon. For each patient, a supplementary blood sample (1 x 25 mL) will be drawn before each cycles of neoadjuvant chemotherapy whatever the treatment arm.

In order to describe the variations of CEC (Circulating Endothelial Cells), VEGF, and its soluble receptor VEGFR2, and endothelial and adhesion cell markers, like P-Selectin, or ICAM-1 levels during neoadjuvant chemotherapy, and the possible differences between the 2 treatment arms with sufficient accuracy; and sufficient statistical power, an increased number of blood sample measures is needed.

For this study, a specific informed consent will be signed by patients before the blood sample.

Centralization and analysis of samples

All blood samples will be sent immediately for analysis to the following address at room temperature**:** S. Ladoire, MD. Medical Oncology. CGFL Dijon.
Samples will be stored for 5 years.

Statistical analysis

Data collected for this biological study will be incorporated into the final database.

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Study 4b: Angiogenesis Imaging

Objectives

Primary objective:
To reliably assess therapeutic response using biological markers as well as functional imaging to evaluate neo angiogenesis modifications in the tumor.

Secondary objective:
To identify imaging criteria allowing the early identification of responders and non-responders patients.

Rationale

Contrast ultrasound with microbubbles injection (Sono Vue, Bracco*), is a simple, non invasive, reproductible technique, to measure tumoral perfusion, and perfusion changes allowing the assessment of tumor vascularization modifications (1-3). Quantification softwares to obtain numerical parameters to precisely quantify tumor flow are used. The accuracy of contrast-enhanced ultrasound for microvascular perfusion measurement and perfusion changes following therapy has been documented both in experimental models (4), and in patients with cancer (5).

Over other functional imaging techniques (like DCE-MRI, or DCE-CT), contrast-enhanced ultrasound present several advantages: measured values strictly refer to the intravascular compartment and are not confounded by extra vascular diffusion; repeat measurements can be taken with equipment that is easily accessible and has limited costs. Moreover, patients are not exposed to ionizing radiation.

Although these promising advantages, contrast-enhanced ultrasound and its impact on early decisions during antiangiogenic treatment remain uncertain. This outcome might change if imaging analysis can be associated (like in our study) with analysis of molecular and biological biomarkers, and compared with PFS and OS to test their value as surrogate markers.

Assessment calendar

The calendar is the same as DCE-US core protocol:
Mammary DCE-US will be performed:

- Before the start of the treatment (at screening) for all patients,
- The following mammaries DCE-US (at C3 and C4) will be only performed for patients of Arms A and B and only if contrast enhancement of the 1st mammary at screening is > 50%. These following mammaries DCE-US must be performed within 7 days before C3 and within 2 days before C4.

Statistical analysis
Data collected for this imaging study will be incorporated into the final database.

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