MONITORING AND PREVENTION OF DRUG CARDIOTOXICITY CAUSED BY ANTHRACYCLINE AND TRASTUZUMAB IN THERAPY OF BREAST CANCER: THE EFFECTS OF TREATMENT WITH ACE INHIBITORS AND BETA BLOCKERS (SAFE STUDIO 2014)

Version of the protocol: 2.2.2014

Summary

Administrative data

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Statement of the principal investigator compliance with the Declaration of Helsinki, with the national legislation and the Protocol

I, the undersigned, Prof. Lorenzo Livi declare to know the study protocol and sponsor me because this is conducted as described and in accordance with:

- principles stated in the Declaration of Helsinki
- Good Clinical Practice
- EU directives and regulations relevant national regulations
- Any changes to procedures will be made exclusively to protect the safety, rights and welfare of subjects involved in the study.
- I declare to coordinate the study by ensuring that all those who collaborate in the execution will be aware of the protocol and any amendments and will act in full awareness of their obligations.

In faith

Data _____

Signature _____

1) Rationale

1.1) Anthracyclines and trastuzumab in the treatment of breast cancer - Effectiveness

Over the years, thanks to the use of therapeutic schemes, of drugs and chemotherapeutic agents of the new generation, as well as the use of radiation techniques increasingly advanced and precise towards the target to be treated, it is able to obtain a high rate of curability of breast cancer with an overall survival after 10 years estimated at approximately l '80%.

Anthracyclines have a key role in the treatment of breast cancer. A randomized multicenter Italian study has demonstrated a benefit of disease-free survival in patients with positive lymph nodes treated with anthracyclines and CMF with respect to the following scheme CMF alone (20). These results are confirmed by the analysis of two similar combined British studies, which indicate a benefit in disease-free survival and overall survival for those who received subsequent chemotherapy with epirubicin and CMF scheme than those who received CMF alone (21). Many are the chemotherapy regimens containing anthracyclines and taxanes in clinical practice in the treatment of breast cancer. Numerous randomized trials have confirmed the benefit of the addition of taxanes to anthracyclines. The last dell'EBCTCG meta-analysis (22) has considered 33 studies that enrolled a total of 44,000 patients; the meta-analysis confirmed a reduction in the risk of relapse by 13% and the risk of death (from any cause) of 11% in favor of taxane-containing regimens.

Trastuzumab is a recombinant humanized monoclonal antibody with specificity for the extracellular domain of HER2. The use of trastuzumab administered sequentially or

concurrently with adjuvant chemotherapy compared to chemotherapy in patients with HER2 positive was evaluated from six randomized trials.

Two studies conducted in North America, NSABP B-31 and NCCTG 9831 (23,24), compared chemotherapy (AC regimen followed by paclitaxel) in combination with trastuzumab versus chemotherapy alone in patients with breast cancer operated with positive axillary lymph nodes or with negative lymph nodes at high risk of relapse (7% of the whole population).

The addition of trastuzumab resulted in a reduction in the risk of recurrence of 12.8% overall and an absolute advantage in overall survival of 3.2% at a median follow-up of 2.9 years. The combined analysis of the two studies has recently been updated with a median follow-up of about 4 years and continues to demonstrate a reduction in the risk of relapse by 52% and death by 39% in favor of the addition of trastuzumab to chemotherapy. In another study conducted in Europe HERA (25) of the patients with positive axillary lymph nodes or negative at high risk were randomized after adjuvant chemotherapy and radiotherapy, to receive trastuzumab every 3 weeks (for 1 or 2 years) or no treatment. Trastuzumab for one year significantly reduced the risk of recurrence. E 'was in fact confirmed at a median follow-up of two years, the benefit in terms of disease-free survival (6.3%) and was also shown a significant benefit in overall survival (2.7%). As regards the administration of trastuzumab for two years total compared to administration of one year, it was not revealed any statistically significant advantage in neither disease-free survival or overall survival in (26).

The study BCIRG 006 (27) compared a regimen containing anthracycline and docetaxel in sequence with the same regimen in combination with trastuzumab administered

concomitantly with docetaxel or with a non-anthracycline regimen (carboplatin and docetaxel) with trastuzumab administered concomitantly. At a median follow-up of 65 months was observed in both regimens a significant advantage in disease-free survival and overall survival with both trastuzumab-containing regimens (with or without anthracycline) compared to chemotherapy and with no significant difference compared to two arms.

The study FINHER (28) instead randomizes patients with node-positive or node-negative high-risk to receive 3 cycles of docetaxel or vinorelbine, followed in both groups by 3 cycles of FEC. The subgroup of women with HER-2 positive disease was further randomized to receive or not receive trastuzumab for a total of nine weeks at the same time as vinorelbine or docetaxel. E 'was shown a statistically significant advantage in relapse-free survival at a distance at a median follow-up of 62 months, while in an exploratory analysis the advantage was statistically significant in the subgroup of patients treated with docetaxel concurrent trastuzumab.

In the last randomized study (PACS-04) (29), the FEC scheme has been compared with the regime epirubicin and docetaxel for 6 cycles with positive lymph nodes. At the end of chemotherapy and radiotherapy for patients with HER 2 positive tumors were randomized to receive or not receive trastuzumab in sequence with chemotherapy for a year. At a median follow-up of 4 years were significant differences in either disease-free survival or in overall survival between the two arms (this is so far the only negative study, but the number of patients was small, about 10% of those randomized in the arm with trastuzumab did not receive the drug.

Overall, the majority of studies conducted with trastuzumab in the adjuvant setting have shown excluding studies with smaller sample (PACS-04 and FINHER), an advantage in disease-free survival and significantly variable from 6% to 12.8% compared to the control with the administration for one year. The advantage in overall survival was instead obtained only with the administration of trastuzumab in combination with chemotherapy (taxane), but not in sequence to it with an absolute advantage varying from 3.2% to 5%.

However, in patients who received Herceptin concurrently with a taxane after anthracycline-containing regimen has been a significant increase in cardiotoxicity.

In the study of Perez (30) has been highlighted as a total of 1576 patients, 745 of these well performed a decrease in LVEF of <15% and a 6.7%, 96 patients, occurrence of a Grade 2 cardiac toxicity. In another recent study by the group of Perez, published in 2008, has proven itself as the association with anthracyclines and cyclophosphamide in the Trastuzumab, leading to an increase of approximately 10% risk of developing cardiotoxicity and in particular ventricular dysfunction left (31). However, trastuzumab administered at the end of all adjuvant treatments such as in the HERA trial, has determined only a slight increase in cardiac events reversible after discontinuation of the drug and appropriate cardiological therapy (32).

1.2) Anthracyclines and trastuzumab in the treatment of breast cancer - Toxicity

Using many years of chemotherapy drugs and biological burdened with cardiac toxicity in the adjuvant and the advanced stage of breast cancer, has made available data on the incidence of adverse cardiovascular events acute, subacute and late, and how to monitor patients.

The cardiac toxicity of anthracyclines may be acute, subacute and chronic. The acute toxicity occurs during or shortly after infusion of the drug with arrhythmias (PSVT), which in some cases leads to heart failure, pericarditis-myocarditis and ECG changes. The acute toxicity is reversible and dose-dependent manner. Subacute replaced after a few weeks with myocarditis, abnormal diastolic function with a mortality rate of 60%. The acute and subacute toxicity are rare (1-4%) . Clinically relevant cardiac toxicity with a chronic progressive deterioration of ventricular function up to heart failure. And 'well known as the main risk factor is the cumulative dose. At the dose is added as risk factors advanced age, female sex, and the combination with cyclophosphamide / taxane / trastuzumab, previous mediastinal radiation therapy, cardiovascular comorbidity. In most cases, the late toxicity occurs within the first year following completion of chemotherapy but nevertheless the clinical manifestations can occur even after 10-20 years; This suggests that in women treated in adjuvant mode is necessary an echocardiographic monitoring even after a longer time.

Approximately 20-25% of breast carcinomas have an overexpression of HER2. For over 10 years, trastuzumab is registered for the treatment of women with advanced breast cancer with HER2 positive and subsequently in adjuvant. In early studies of association with anthracyclines, the incidence of severe heart failure (NYHA III-IV) was 16%. In monotherapy the same severe cardiac toxicity was reduced to 2%. The pathogenetic mechanism appears to be related to a blockade of the HER2 signal in the myocyte with a block of the street to protect the myocardial cell. This toxicity is reversible (1). A meta-analysis of 2011 has further analyzed the toxicity of trastuzumab in breast cancer patients treated at an advanced stage and

adjuvant. Out of 11,882 patients treated in 10 randomized trials to reduce the incidence of ventricular function was 7.5% and 1.9% of heart failure with an increased risk reduction of LEVF. The increased risk was significant in patients treated with anthracyclines compared to non-treated (2). A retrospective cohort study published in 2012 in the Journal of the National Cancer Institute shows that the adjusted risk of failure and / or cardiomyopathy is increased 4-fold in women treated with trastuzumab monotherapy and 7-fold in those treated with anthracyclines and trastuzumab compared women not undergoing chemotherapy. Overall, the risk of failure and cardiomyopathy associated with the use of anthracyclines among women under 65 years is similar to that reported in randomized clinical trials, while that associated with the use of trastuzumab monotherapy administered or after an anthracycline is appeared greater than that previously appeared in (3). With cardiotoxicity means a reduction of the FE of greater than 10 percentage points, during the chemotherapy treatment, associated with a reduction below the normal limit (50%). During the follow-up is recommended an assessment of LVEF at 3,6,9,12 months after the end of chemotherapy. In practice, this evaluation is performed with echocardiography, but this method does not seem to be sufficiently sensitive and specific in predicting the development of cardiac dysfunction after chemotherapy. For this it is important to have the tools that allow you to identify the patient who most likely will encounter cardiac dysfunction is present even before this. One of these tools is the TnI. In literature there are several studies that have examined how the monitoring of TnI, soon after the end of infusion of chemotherapy drugs, may provide important information in terms of prognostic and diagnostic (4,5). The trastuzumab

cardiotoxicity is reversible, but reversibility, however, is not predictable and the identification of patients at risk and those who did not recover cardiac function is critical. In Study by Dr. Cardinal (6,7) enrolled 251 patients in whom we measured the levels of TnI before and after each cycle of trastuzumab. FE assessed at baseline, every 3 months during trastuzumab therapy and then every 6 months. In the case of cardiotoxicity from trastuzumab therapy was stopped and started the therapy of heart failure with enalapril and carvedilol. Cardiotoxicity from trastuzumab occurred in 42 patients (17%) and was more frequent in pieces with increased TnI, in 25 patients (66%) cardiotoxicity was resolved and the resolution of the FE occurred less frequently in patients with increase of TnI; multivariate analysis, the increase in TnI was found to be the only independent predictor of cardiotoxicity from trastuzumab. So in conclusion, the increased levels of TnI allows to identify patients treated with trastuzumab who are at risk of cardiotoxicity and are less likely to restore cardiac function despite treatment of heart failure (8). A similar study was also performed for anthracyclines with the objective of evaluating the change early, and 2 years of FE and the effects of chemotherapy on TnI and BNP levels. The study showed that the FE and BNP values have changed so early after treatment with anthracyclines: 2 years after chemo the FE has not returned to the initial values (9).

Recent studies have taken into account new markers of chronic heart failure as the Galectin-3 sst2 and which therefore could also be used as monitoring of cardiotoxicity from chemotherapy. The plasma levels of galectin-3 appear to be related to changes in the structure and left ventricular function indicating how this new serological marker may be

involved in the process of left ventricular remodeling in chronic heart failure. The specificity of galectin-3 in predicting the development of heart failure appears to be higher in the NT-proBNP, but not the sensitivity. (10) The levels of sst2 (soluble ST2) were assessed in a group of hypertensive patients with stable chronic heart failure with normal FE. The study concludes that the measurement of SST2 provides diagnostic support for stable chronic heart failure with normal FE for hypertensive patients. The addition to the NT-proBNP assay sst2 could give more information regarding the functional class HF and diastolic dysfunction (11).

In addition to serological markers as reported in a recent review of 2013 other early markers of cardiotoxicity are represented by echocardiographic parameters. Most of the data concern as mentioned above systolic dysfunction, few data on diastolic dysfunction, an increasing number of jobs that would suggest instead that the tissue Doppler and strain can reveal changes in pre-clinical contractility, and myocardial deformation in patients with trastuzumab (review journal of the American college of cardiology) (12,13)

With regard to the prevention and treatment of cardiotoxicity from trastuzumab, there are currently proven strategies. Pituskin and col. We propose to explore with the study Manticore 101- BREAST potential role of conventional drug therapy of heart failure in preventing ventricular remodeling as assessed by MRI, induced by trastuzumab in patients with breast cancer HERb2 +. In this randomized, placebo-controlled, double-blind, will be enrolled 159 k pcs with HER2 + breast cancer. Participants will be randomized to treatment with an ACE-I (perindopril), a beta-blocker (bisoprolol) or placebo. The patients will carry the medication for a year, beginning 7 days before the first trastuzumab. The drugs will be titrated to the

maximum tolerated dose at intervals of 1 week, for a total of 3 weeks in all study groups. The main objective is to determine whether the failure of conventional drug therapy can prevent left ventricular remodeling induced by trastuzumab in early breast pieces with k HER2, evaluating the change in volume of the left ventricle at end diastole to 12 months of therapy via resonance. (14). A 2012 review has reported a low percentage of symptomatic heart failure induced by trastuzumab, but a high incidence of non-symptomatic left ventricular dysfunction. Once treatment with trastuzumab is interrupted, standard therapy of heart failure is effective. ACE-inhibitors, beta-blockers and sartans can be used in patients with early signs of heart failure revealed by serological markers and echocardiographic parameters (13). A recent review and metanalysis of 2013 examines several studies that have investigated the possible role of cardioprotective therapy in preventing cardiac toxicity of anthracyclines and trastuzumab. The drugs taken into consideration were beta-blockers, ACE-inhibitors, statins and dexrazoxane, and all seem to be equally effective in reducing cardiac toxicity of chemotherapeutic drugs (15).

Seicean and coll. have examined in a study of May 2013 the effect of beta-blockers therapy in women with breast cancer treated with anthracyclines and trastuzumab. The study showed that the use of beta-blockers is associated with a low incidence of heart failure in patients with breast cancer and a normal baseline ejection fraction (16,17).

A recent work of 2012 (J Cardiol 2012 Jun 22) (18) has evaluated the efficacy of nebivolol in preventing anthracyclines-induced cardiac toxicity in breast cancer patients, monitoring echocardiographic parameters, TnI and BNP. This study demonstrates how preventive use of

the beta-blocker can protect breast cancer patients from chemotherapy-induced cardiac toxicity.

Use of statins in breast cancer patients before and after chemotherapy with anthracyclines is also associated with a lower risk of new-onset heart failure. This is demonstrated in a retrospective study published in the Journal of the American College of Cardiology (19). The authors have examined 628 women with breast cancer treated with anthracyclines. The primary outcome represented by hospitalization for new-onset heart failure was evaluated comparing patients that received statins continuously during an average follow-up period of 2,5 years with patients that did not receive statins continuously. Both mortality due to heart failure and mortality due to cancer were significantly lower in the group treated with statins, with only 4 cases of heart failure in the group treated with statins and 23 in the control group. Moreover 15 deaths correlated to cancer were registered in the group not treated with statins, while no decease was observed among the women treated with statins. The authors believe that women with higher risk of anthracyclines-induced cardiac toxicity should be taken into consideration for statins therapy, especially those with hypertension, coronary disease, and combined use of trastuzumab and mediastinal radiation therapy.

Seican S et al (33) have recently demonstrated that continuous therapy with beta-blockers is associated with a significantly lower incidence of heart failure in patients with breast cancer treated with anthracyclines chemotherapy and trastuzumab, and with a normal baseline ventricular ejection fraction. The authors hope for new prospective phase 3 studies to confirm these results.

In particular, the cardio-oncologic scientific community increasingly seems to be looking forward to a routine cardioprotection therapy for patients with breast cancer with indication for adjuvant therapy involving anthracyclines and/or trastuzumab (34-37).

2) Aim of the study

The aim of the study is to analyze the protective capacity on the cardiac damage of breast cancer therapy with anthracyclines and trastuzumab, of drugs known to be effective in other forms of overt heart failure, in particular of cardioselective betablocker and ACE-inhibitor, alone or in association.

2.1) Primary endpoint

Evaluation of the reduction of subclinical cardiotoxicity incidence in women treated compared to the placebo group.

2.2) Secondary endpoints

Evaluation of reduction of both systolic and diastolic, early and late cardiac damage, measured with conventional echocardiography, pulsed tissue doppler and measurement of some myocardial toxicity biomarkers.

2.3) Inclusion criteria

Breast cancer patient (any histological type) at early stage

Indication to treatment with adjuvant anthracyclines +/- trastuzumab

Informed consent

Age ≥ 18 years

2.4) Exclusion criteria

Previous treatment with anthracyclines, ACE inhibitors, beta-blockers, or sartans

Baseline ventricular ejection fraction of <50%; baseline positivity of biomarkers

Previous solid tumor treated with systemic therapy

Recurrence of breast cancer

Age <18 years

Pregnancy

3) Patients and Methods

3.1) Treatment Groups

The study considers 4 groups, all treated with one of the chemotherapy schedules allowed for the study (including anthracyclines and/or trastuzumab):

Group 1 (Placebo 1 + Placebo 2): 1 tablet in the morning and 1 tablet in the evening, until the end of the study.

Group 2 (Bisoprolol + Placebo 2): 1 tablet of placebo in the morning and 1 tablet of Bisoprolol 5 mg in the evening, starting 1 week before chemotherapy treatment.

Group 3 (Ramipril + Placebo 1): 1 tablet of placebo in the morning and 1 tablet of Ramipril 5 mg in the evening, starting 1 week before chemotherapy treatment.

Group 4 (Bisoprolol + Ramipril): 1 tablet of Ramipril 5 mg in the morbing and 1 tablet of Bisoprolol in the evening, starting 1 week before chemotherapy treatment.

Drugs will be obviously suspended if symptomatic bradycardia, hypotension, cough or other drug intolerance occur.

3.2) Clinical Assessments

Time 0: Medical history, clinical examination, ECG, routine blood tests, sampling for troponin-I, NT-proBNP and sST2. Conventional echocardiography and pulsed wave tissue Doppler echocardiography.

Time 1 (3 months): clinical examination, ECG, routine blood tests, sampling for troponin-I, NT-proBNP and sST2. Echocardiography and pulsed wave tissue Doppler echocardiography.

Time 2 (6 months): clinical examination, ECG, routine blood tests, sampling for troponin-I, NT-proBNP and sST2. Echocardiography and pulsed wave tissue Doppler echocardiography.

Time 3 (12 months): clinical examination, ECG, routine blood tests, sampling for troponin-I, NT-proBNP and sST2. Echocardiography and pulsed wave tissue Doppler echocardiography.

Time 4 (24 months): clinical examination, ECG, routine blood tests, sampling for troponin-I, NT-proBNP and sST2. Echocardiography and pulsed wave tissue Doppler echocardiography.

3.3) Statistical Analysis

Incidence of cardiac toxicity in breast cancer patients treated with chemotherapy is highly variable, especially in relation to patient's age, comorbidities, as well as dose, type and schedule of the used chemotherapy drug. Some studies have reported an incidence ranging between 5% and 65% (Swain SM et al, Cancer 2003). The incidence of cardiotoxicity following the use of trastuzumab alone and/or in combination with other chemotherapy drugs in HER2-positive breast cancer is as much as variable (from 4% to 55%) (Slamon DJ et al, NEJM 2001; Yoon GJ et al, J Am College Cardiology, 2010).

The proposed study is a randomized, double blind trial using a 2x2 factorial design, in which patients are randomly allocated to receive either one of the two types of treatment, no intervention, or both. The participants are assigned to the trial arms as follows:

		TREATMENT A (bisoprolol) YES	NO
TREATMENT B (ramipril)	YES	A+B	B
NO		A	No intervention

The reported rate of subclinical cardiotoxicity measured with traditional methods and tissue Doppler imaging varies in a range of 20–75%. Considering a median rate of subclinical alteration of 40%, with 15% reduction in treatment groups to reach the outcome, the study design required a sample of 90 patients per treatment group provided an 80% statistical power. The interim analysis statistics is based on an anticipated 11% change in EDVI within-group, standard deviation of 20 mL, a two-tailed significance level of a = 0.05, and 80% power required 47 patients per group.

The primary **outcome** of the study is reduction of subclinical cardiotoxicity incidence among treated compared to non-treated women at 12 and 24 months of follow-up, in order to determine whether treatment with bisoprolol, ramipril or the combination of both drugs can prevent or reduce the cardiotoxic effects in patients treated with adjuvant therapy for breast cancer. One major advantage of the factorial design is that it allows to investigate at the same

time the two treatments including all participants in both analyses, and to consider the distinct effects of each intervention and the benefits of both as well.

The allocation of participants in the trial arms will be carried out through a **stratified randomization**, using randomized permuted-blocks within defined strata according to age and HER2 status, obtained with R software. Randomization will be performed by an operator external to the study. All clinicians and researchers involved, and the same patients, will not know to which arm each patient will be assigned.

All statistical analyses, based on the comparison of different groups, will be conducted according the *intention to treat* analysis principles.

Simple descriptive statistics will be used to compare individual characteristics of patients who are allocated between distinct groups at *time 0* (*baseline*). For this purpose appropriate statistical tests will be used, such as chi-squared test for categorical variables and the Mann-Whitney U test for continuous variables.

As a *primary analysis*, the estimation of efficacy of a given intervention will be made by comparing all the subjects allocated to receive that intervention with those allocated to not receive that intervention. For example, the intervention with bisoprolol will be evaluated comparing the two groups with this intervention (bisoprolol alone + bisoprolol and ramipril) with the other two groups (ramipril alone + placebo). These comparisons will be made by multivariate regression models adjusting for other possible confounding variables (covariates).

Although the size of the study does not allow investigations over possible interactions between the two interventions, these effects will be possibly investigated as a *secondary analysis* with multivariate regression models inserting an appropriate interaction term.

Appropriate descriptive statistics will also be used to describe different individual characteristics measured at different times of follow-up (e.g. 6, 12, 24 months) within each of the 4 factorial cells of the trial.

Trial results will be reported as different esteems with confidence intervals of 95% comparing subjects allocated to receive a given intervention with the subjects allocated to not receive that intervention.

Values of $p<0,05$ will be considered statistically significant. All statistical analyses will be conducted using IBM software SPSS Statistics (version 22).

3.3) Cardiological clinical assessment

Medical history, ECG, clinical examination with specific attention to signs of heart failure, NYHA class registration, Canadian angina grading scale score. Blood samples with measurement of biohumoral markers (Troponin and NT-proBNP, galectin, sST2).

Echocardiographic assessment: evaluation of heart chambers dimensions with specific attention to left and right ventricular remodeling.

Study of left ventricular systolic and diastolic function with conventional procedure (Ejection fraction calculated with Simpson Biplana, Fractional Shortening in M-mode, evaluation of left ventricular filling by pulsed Doppler of mitral inflow).

Study of left ventricular systolic function by pulsed Tissue Doppler procedure of mitral annulus and by 2D-speckle tracking (systolic mitralic annulus excursion evaluated with pulsed Tissue Doppler, left ventricle longitudinal strain evaluated with 2D speckle tracking from apical views).

Evaluation of left ventricular diastolic function with Pulsed Tissue Doppler (evaluation of diastolic mitralic annulus excursion Em and its relation to the E wave of mitralic filling).

Evaluation of right ventricular systolic function with conventional procedure (tricuspidal plane excursion, TAPSE), with pulsed Tissue Doppler (systolic tricuspidal plane excursion) and with 2-D Speckle Tracking (right ventricular free wall longitudinal strain).

Evaluation of right ventricular diastolic function with conventional procedure (pulsed Doppler of tricuspidal inflow) and by pulsed Tissue Doppler (tricuspidal plane Em).

Diastolic damage is defined as a worsening of diastolic function by at least one class from baseline, according to the current classification based on the mitral inflow at pulsed Doppler (impaired relaxation pattern, pseudonormal filling pattern, restrictive pattern).

Systolic damage is defined according to literature (38), as a reduction of EF >5% from baseline and an absolute value of <55% with onset of heart failure symptoms, or a reduction of EF >10% and an absolute value <55% with no symptoms.

An early cardiac event is defined as an event occurring within 12 months from the beginning of therapeutic protocol.

A late cardiac event is defined as an event occurring after 12 months from the beginning of therapeutic protocol.

The examinations will be conducted in the same ECHO-lab with the same machine and by the same operator.

Evaluation of reduction of the symptomatic heart failure incidence, considering not hypnotizable a cardiopulmonary test in all patients, will be based on NYHA functional evaluation and generally on the onset of heart failure signs and/or symptoms (such as peripheral edema, dyspnea, jugular turgescence).

3.4) Chemotherapy schedules used in pre-operatory/post-operatory setting

- **FEC+TXT 100: Epirubicin** (100 mg/mq), **Cyclophosphamide** (500 mg/mq), **5-FU** (500 mg/mq, 3 cycles every 21 days) + **Taxotere** (100 mg/mq, 3 cycles every 21 days);
- **FEC+TXT 75**: **Epirubicin** (75 mg/mq), **Cyclophosphamide** (500 mg/mq), **5-FU** (500 mg/mq, 3 cycles every 21 days) + **Taxotere** (75 mg/mq, 3 cycles every 21 days);
- **FEC 100**: **Epirubicin** (75 mg/mq), **Cyclophosphamide** (500 mg/mq), **5-FU** (500 mg/mq, 6 cycles every 21 days);
- **AC*:* Doxorubicin** (60 mg/mq), **Cyclophosphamide** (600 mg/mq, 4 cycles every 21 days);
- **EC : Epirubucin** (75 mg/mq), **Cyclophosphamide** (600 mg/mq, 4 cycles every 21 days);
- **AC/EC + TAX: AC** or **EC** (4 cycles every 21 days) + **Taxolo** (175 mg/mq, 4 cycles every 21 days, OR 80 mg/mq, 12 cycles weekly);
- **EC "dose dense": Epirubucin** (75 mg/mq), **Cyclophosphamide** (600 mg/mq); 4 cycles every 15 days, with primary prophylaxis with G-CSF;

- Every Anthracyclines based schedule can be followed by **Trastuzumab** (8 mg/Kg loading dose; 2 mg/mq maintenance dose, weekly), for 18 cycles every 21 days;
- **Trastuzumab** can be administered in association with **taxolo** or **taxotere**, every 21 days or weekly (4 mg/Kg, loading dose; 2 mg/mq, maintenance dose, weekly).

3.5) Dose Reductions

-EPIRUBICIN dose reduction: in patients with impaired hepatic function, dose must be reduced on the basis of serum bilirubin, as it follows: serum bilirubin 1,4-3 mg/100 ml, AST (aspartate aminotransferase) 2-4 times higher than uppermost normal value: dose reduction of 50%. Serum bilirubin >3 mg/100 ml, AST (aspartate aminotransferase) >4 times normal value: dose reduction of 75%. Impaired renal function: no dose reduction seems to be required considering the small amount of epirubicin excreted through this way. However, in patients with serum creatinine >5mg/dl dose adjustment could be necessary.

-CYCLOPHOSPHAMIDE dose reduction: leukocyte count 4000-2500 o platelet count 100000-50000 expect a 50% reduction of the programmed dose; <2500 or <50000 expect values normalization or medical decision; A dose reduction of 25% is recommended for serum bilirubin values between 3,1 and 5 mg/100 ml and a 50% reduction for a glomerular filtration rate less than 10 ml/min.

-DOXORUBICIN dose reduction: patients with impaired hepatic function: serum bilirubin 20-50 mcmol/l, half of the normal dose; serum bilirubin between 50 and 85 mcmol/l, a quarter

of the normal dose; serum bilirubin >85 mcmol/l, treatment interruption. Patients with impaired renal function: in patients with renal failure (GFR<10 ml/min) only 75% of the programmed dose must be administered. In case of pre-existing cardiopathy, or previous cardiac mediastinal irradiation a cumulative dose > 400 mg/mq must be avoided. A cumulative dose of 450-550 mg/m^2 must be avoided.

-TAXOTERE dose reduction: patients that have developed, during docetaxel treatment, febrile neutropenia, neutrophil count <500 cells/mm^3 for more than a week, serious or cumulative cutaneous adverse effects, or severe peripheral neuropathy, docetaxel dose must be reduced from 100 mg/m^2 to 75 mg/m^2 and/or from 75 to 60 mg/m^2. Patients presenting with Grade 3 or 4 stomatitis: dose must be reduced to 60 mg/m^2. In patients that develop a second occurrence of Grade 2 toxicity, or a first occurrence of Grade 3 toxicity at any time during therapy, treatment must be suspended until resolution to Grade 0-1, and continued at 55 mg/m^2. In case of any further occurrence of toxicity, or any Grade 4 toxicity, interrupt treatment. If additional episodes of complicated neutropenia occur docetaxel dose must be reduced from 60 to 45 mg/m^2. In case of Grade 4 thrombocytopenia docetaxel dose must be reduced from 75 to 60 mg/m^2. Patients must not be treated with other docetaxel cycles until a neutrophil count >1500 cell/mm^3 and platelets >100000/mm^3.

-TRASTUZUMAB dose reduction: patients with early breast cancer and a left ventricular ejection fraction (LVEF) equal or less than 55%, should be treated carefully. If LVEF decreases by 10 ejection fraction (EF) points from baseline and is less than 50%, treatment must be

suspended and LVEF evaluated again after about 3 weeks. If LVEF does not improve or further reduces, treatment interruption must be seriously taken into consideration.

Management of adverse events

Adverse events will be reported in according with the instructions in the national reference standard legislation (Legislative Decree 211/2003).

Adverse Events / Adverse Reactions (AE / ADR)

"Adverse event" means any untoward medical event that occurs in a patient or a person involved in a clinical trial and who has been administered a medicinal product, and which does not necessarily have a causal relationship with this treatment;

"Adverse reaction": any untoward and unintended reaction to an investigational medicinal product regardless of the dose administered.

"Serious adverse event or serious adverse reaction": any untoward medical occurrence or effect that, regardless of the dose administered, causes death or has endangers the life of the patient, requires hospitalization or prolongation of hospitalization, or that determines severe or prolonged disability or incapacity, or congenital anomaly or involves a birth defect.

“Unexpected adverse reaction "means an adverse reaction which nature or severity is not predictable based on information concerning the product (for example, those described in the “Investigator's Brochure” if the product is in the trial or, in the case of an authorized product, the tab of the product characteristics).

An adverse event is "any untoward clinical event that occurs in a patient or a person involved in a clinical trial who was administered a medicinal product and which does not necessarily

have a causal relationship with this treatment. An adverse event (AE) can therefore be any sign (including an abnormal laboratory finding), unfavorable or unintended, symptom or disease temporally associated with the use of the medicinal product (in testing) for the coincidence in time, whether related or not to the medicinal product (on trial) "(Note for Guidance for Good Clinical Practice" CPMP / ICH / 135/95).

Any person, who will show an adverse event, will be examined by a doctor as soon as possible. The doctor will do what is necessary for the safety and well-being of the subject. All anomalies will be followed until healing or clinical stabilization.

The adverse event will be described in medical records using standard medical terminology (MedDRA) to avoid the use of vague expressions, ambiguous or colloquialisms. The investigator will examine all adverse events with regard to the gravity and the relationship with the investigational product and report the results of the analysis and actions to be taken. "All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The expression "responses to a medicinal product" means that there is at least a reasonable possibility of a causal relationship between a medicinal product and an adverse event, namely, which the relationship cannot be ruled out. "(Note for Guidance for Good Clinical Practice" CPMP / ICH / 135/95).

<u>Adverse Events / Serious Adverse Reactions (SAE / SADR)</u>

A serious adverse event / serious adverse reaction are "any untoward medical occurrence or effect that, at any dose:

causes death

endangers the life of the patient

requires hospitalization or prolongation of hospitalization

causes serious or prolonged disability or incapacity

involves a congenital anomaly or birth defect. "

(Note for Guidance for Good Clinical Practice "CPMP / ICH / 135/95).

A not serious adverse event /reaction is an AE / ADR that does not meet the above criteria.

Unexpected adverse reactions

An unexpected adverse reaction is an "adverse reaction whose nature or severity is not predictable based on information concerning the product (for example, those described in the Investigator Brochure if the product is on trial or, in the case of an authorized product, in summary of product characteristics). "(Note for Guidance for Good Clinical Practice "CPMP / ICH / 135/95).

Methods of reporting

Serious adverse events / serious adverse reactions

The investigator has to notify the promoter immediately, but not later than 5 calendar days, any adverse event (AE) occurring during the study, filling the appropriate field reserved in the medical record; will follow a detailed written report to be sent to the promoter within 3 calendar days from the first alert.

All fatal or life-threatening AE must immediately be reported to the promoter submitting a "Form for Urgent Report ", not later than 24 hours from the moment in which the experimenter gets to know the AE. Will follow, in the subsequent 48 hours, a full and detailed report of the case.

The promoter ensures that all relevant information about suspected serious unexpected fatal or life-threatening adverse reactions will be recorded and reported as soon as possible to the Ministry of Health, and to the involved Ethics Committee, within 7 calendar days from the moment when the promoter is alerted and awarded of the case, and that subsequent relevant information will be communicated within 8 days from first report.

Any serious adverse events must be reported to the Promoter by fax to the following contacts:

Prof. Lorenzo Livi / Dr. Icro Meattini fax 055/79479363

Any other suspected serious unexpected adverse reactions (non-fatal or life-threatening) will be notified to the Ministry of Health and to the involved Ethics Committee, as soon as possible and, in any case, within 15 days from the date on which the promoter of the experimentation gets to know about the fact.

For all the duration of the clinical trial, once a year, the promoter will provide the Ministry of Health and the involved Ethics Committee a list of all suspected serious adverse reactions observed during this period and a report on the safety of patients involved in the clinical trial.

<u>Non-serious adverse events</u>

The investigator has to report to the promoter any serious adverse events. The information will be collected in the medical record.

Instructions for the study staff about severe unusual signs or symptoms

Each subject will be informed who to contact in case of any severe or unusual signs or symptoms that will appear after treatment. The persons who may experience severe systemic reactions, if possible, should be visited in the institute at the time of maximum expression of symptoms and will be followed clinically until resolution.

Any event that causes the withdrawal of the subject from the study will be reported in the appropriate section of the medical record. All adverse events will be followed until the healing and / or diagnosis. If an adverse event is not resolved at the conclusion of the study, the investigator will decide whether to authorize the follow-up. All medications taken during the study to treat adverse events or previous diagnosis will be recorded in the medical record.

In the case where it is possible to formulate a diagnosis, it is preferable to give the diagnosis rather than using a series of terms related to it. When you report a syndrome, indicate the signs and symptoms associated as part of the syndrome, not as separate events.

<u>Causal connection</u>

The investigator will evaluate the association between adverse events and treatment according to the following definitions:

Certain

A clinical event - including laboratory test abnormality - which follows, with a reasonable time sequence, the administration of the drug but that cannot be explained by concurrent disease or other drugs. The reaction must have already been observed for the suspected drug. The reaction should improve with the "dechallenge" and reappear with the "rechallenge."

Likely

A clinical event - including laboratory test abnormality - which follows, with a reasonable time sequence, the administration of the drug but that may not be explained by concurrent disease or other drugs, and whose response to "dechallenge" is clinically acceptable. The data of the "rechallenge" are not required.

Possible

A clinical event - including laboratory test abnormality - which follows, with a reasonable time sequence, the administration of the drug but that may also be explained by concurrent disease or other drugs. The data related to discontinuation of the drug may be missing or uncertain.

Unlikely

A clinical event - including laboratory test abnormality - whose temporal sequence of drug administration makes a causal relationship improbable, and where, other medications or underlying disease provide plausible explanations.

Not Classified

A clinical event for which there is insufficient information at the time of the investigation, and for which more data are needed for a proper assessment.

Not Descript

A clinical event for which the information received is inadequate and / or contradictory and do not allow a reasonable assessment.

Abnormalities in laboratory parameters

The investigator should evaluate the clinical significance of these outlier's laboratory parameters in accordance with the definition of default values of reference laboratories. Any clinically significant abnormality must be fully investigated. By "clinically significant" means any anomaly that, according to the investigator, is a significant clinical problem that requires medical intervention or that otherwise meets the definition of a "severe" adverse event. When clinically indicated, you have to make further analyzes or evaluations to determine the significance or the etiology of an abnormal result or to monitor the course of an adverse event. Any persistent outlier parameter must be followed at the discretion of the investigator. The clinically significant abnormal findings will be documented in an specific form of the medical record.

Concomitant diseases and pre-existing illnesses

Concomitant diseases (including signs / symptoms of a pre-existing medical condition) that are present during or before the administration of the investigational product and which occur with the same severity, frequency, or duration after administration of the study drug, should be reported on appropriate form of the medical record. However, cases that show an increase

in the severity or duration of concomitant or pre-existing disease should be reported as adverse events.

Ethical aspects and respect of confidentiality

Investigators ensure that the study will be conducted in full compliance with the provisions of the international regulations [Dir. EU 2001/20 / EC] and its national transposition [DM 15 July 1997; D.Lvo 211/2003; DLvo 200/2007] about the clinical trial and the principles of the Declaration of Helsinki in order to ensure maximum protection of those involved. The Principal Investigator is committed to ensure that the study is conducted in accordance with what is written in this protocol and in the Good Clinical Practice (GCP). The promoter of the study is committed to the protection of sensitive personal data, clinical and otherwise, of the subjects involved in the study, as defined by the national legislation [D.Lvo. 196/2003].

Informed Consent

The investigators, or persons acting on their behalf, are responsible for obtain the informed consent of patients, after adequate information of the patient, about the aims, methods, anticipated benefits and foreseeable risks of the study. The experimenters, or those in charge, must also inform participants that the non-participation or termination of the same will not result in injury or damage to them.

Ethics Committee and Competent Authorities

The promoter will provide to the Ethics Committee of reference and to the Competent Authorities (General Manager AOU Careggi) the study protocol and any other related document provided to the patient (Leaflet and Informed Consent Form). The approval of the Ethics Committee and the Competent Authority shall be obtained before the start of any procedure related to the study and must be documented through official communication to the experimenter. If during the course of the study, changes may be necessary to study protocol, the promoter shall submit to the Ethics Committee an appropriate reference request for an amendment to the Protocol, whose approval will follow the procedures set out in Regulation Ethics Committee.

Data ownership

Data ownership, being an independent study in accordance with the DM December 17, 2004, belongs to the promoter of the Study (DM 17 December 2004, Art. 1, paragraph 2, letter c).

Final report and publication of results

In accordance with ICH-GCP, the Head of the Study is committed to produce a report on the study, publish all the collected data, as described in the Protocol, and to ensure that the data will be reported responsibly and consistently. In particular, the publication of data resulting from this study will take place regardless of the results obtained. The transmission or dissemination of the data, by means of scientific publications and / or presentation in conferences, meetings and seminars, participation in multi-center studies, will be carried out exclusively as a result of purely statistical processing of the same, or however completely

anonymous. Head of the research and the processing of the data is Prof. Lorenzo Livi, charge of the study.

Independence of the study

The Study has all the necessary requirements according to the DM December 17, 2004 (article 1, paragraph 1 and 2) for the definition of "clinical trial aimed at improving clinical practice as an integral part of health care and not for industrial purposes."

Contributions and conflict of interest

Responsible for the study: Prof. Lorenzo Livi, Prof. Roberto Tarquini, Dr. Icro Meattini conceived and drafted this protocol.

Additional Charges

No one.

Sources of contributions

This trial was conceived independently of any commercial organization and will be coordinated, managed and analyzed in independent form. There are no additional costs.

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