

A phase II study of metronomic oral chemotherapy with cyclophosphamide plus capecitabine combined with trastuzumab in advanced breast cancer (GOIM 2905)

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I have read this protocol and agree to conduct this trial in accordance with all stipulations of the protocol and in accordance with the Declaration of Helsinki.

Name of Principal Investigator:

Signature:

Date

Name of Co-investigator:

Signature:

Date

Name of Co-investigator:

Signature:

Date

Name of Co-investigator:

Signature:

Date

Name of Co-investigator:

Signature:

Date

STUDY SUMMARY

BACKGROUND

Preclinical studies have demonstrated an antiangiogenic action, with effective tumor control, for some chemotherapeutic agents when administered frequently at low doses ('metronomic' schedule); this activity is further enhanced by the association with a specific antiangiogenic drug. In clinical trials, metronomic chemotherapy with low dose oral cyclophosphamide and methotrexate (CM regimen) has been shown to be active and well tolerated in advanced breast cancer, yielding an objective response rate of about 20% and a clinical benefit (objective response and stable disease for at least 24 weeks) of about 40%, in the absence of serious toxicity, with a marked drop in circulating VEGF. Recent published data from a phase II trial with the combination of the Capecitabine plus cyclophosphamide regimen plus bevacizumab (a humanized monoclonal antibody against VEGF) have shown an high clinical benefit rate in untreated breast cancer patients. Capecitabine is an oral chemotherapeutic agent, which is converted to fluorouracil particularly within tumor cells. Its daily administration mimics the activity of a continuous intravenous infusion of fluorouracil, which has been shown to exert antiangiogenic activity in preclinical models. For its pharmacokinetic and toxicological features, capecitabine seems particularly suitable for metronomic administration. The available data on the combination of capecitabine and oral cyclophosphamide show a good activity and tolerability.

Trastuzumab (Herceptin®; Genentech, South San Francisco, CA), a recombinant humanized anti-erbB2/HER-2 monoclonal antibody (MoAb) used in erbB2-overexpressing breast carcinoma, has been shown to have antiangiogenic properties. Overexpression of HER2 in human tumor cells is closely associated with increased angiogenesis and expression of VEGF. Indeed, when the VEGF pathway is inhibited, tumor growth is suppressed. The anti-HER2 blocking antibody trastuzumab has been shown to inhibit tumor cell growth and VEGF expression. Thus, potential upregulation of VEGF in cancer epithelial cells likely supports angiogenesis, sustaining and promoting survival and metastasis of tumor cells. Trastuzumab can induce normalization and regression of the vasculature in an experimental human .

In a previous small series, low-dose, oral cyclophosphamide and methotrexate combined with trastuzumab have been shown to have substantial efficacy in metastatic HER2 positive breast cancer and provided a control of the disease (clinical benefit) in a significant proportion of patients. The observed clinical benefit (RP plus RC plus SD for ≥ 24 weeks) in all patients and in patients with disease resistant to previous trastuzumab therapy were 46% (95% CI, 24-68%) and 27% (95% CI, 6-61%), respectively.

In the present study we want to assess the activity and tolerability of a new metronomic regimen with cyclophosphamide + capecitabine (CC), when combined with trastuzumab in Her2 positive untreated metastatic breast cancer patients.

AIM AND DESIGN: this is a single arm phase II study, assessing the activity and tolerability of a metronomic regimen with oral cyclophosphamide + capecitabine in combination with intravenous trastuzumab. The main objective is to assess the activity in terms of overall clinical benefit, defined as the objective response rate plus the rate of stable disease lasting longer than 24 weeks. Secondary endpoints are to describe the toxicity and to estimate the time to disease progression and the overall survival, as well as to evaluate the impact of treatment on tumor angiogenesis, assessing the changes in levels of circulating endothelial cells.

PATIENT POPULATION

Eligibility Criteria

- Pre- or post-menopausal women with histologically proven, locally advanced (inoperable) or metastatic breast carcinoma.
- Measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan.
- No previous lines of chemotherapy for advanced disease (primary and/or adjuvant chemotherapy are allowed, as well as any prior endocrine treatment; prior primary and/or adjuvant trastuzumab therapy are allowed, if ended ≥ 6 months before. At least 4 weeks must have elapsed since prior chemotherapy or radiation therapy, 6 weeks if the last regimen included mitomycin C).
- Age ≥ 18 years and < 80 years.
- Life expectancy of greater than 6 months.
- ECOG performance status ≤ 2 (Karnofsky $\geq 60\%$; see Appendix A).
- Normal organ and marrow function (leukocytes $\geq 3,000/\mu\text{L}$, absolute neutrophil count $\geq 1,500/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$, total bilirubin within normal institutional limits, AST(SGOT)/ALT(SGPT) ≤ 2 x institutional upper limit of normal, creatinine within normal institutional limits or creatinine clearance ≥ 60 mL/min/1.73 m²).
- Geographically accessible for follow up.
- Women of child-bearing potential must agree to use adequate contraception prior to study entry and for the duration of study participation.
- Ability to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

- Presence of cerebral or leptomeningeal involvement.
- Previous or concomitant other malignancy except basal or squamous cell carcinoma of the skin or adequately treated in situ carcinoma of the cervix.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to trastuzumab or other agents used in the study.
- Serious cardiac illness or medical conditions including but not confined to:
 - History of documented congestive heart failure (CHF) or systolic dysfunction (LVEF $< 50\%$)
 - High risk uncontrolled arrhythmias (ventricular tachycardia, high grade AV-block, supraventricular arrhythmias which are not adequately rate-controlled);
 - Angina pectoris requiring antianginal medication
 - Clinically significant valvular heart disease
 - Evidence of transmural infarction on ECG
 - Poorly controlled hypertension (systolic > 180 mmHg or diastolic > 100 mmHg)
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, renal failure, or psychiatric illness/social situations that would limit compliance with study requirements.
- Pregnant women are excluded from this study because of the potential for teratogenic or abortifacient effects of trastuzumab and other agents included in this trial. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother, breastfeeding must be discontinued.

TREATMENT SCHEDULE

cyclophosphamide, one 50 mg tablet daily (at 9 AM), plus
capecitabine, three 500 mg tablets daily after lunch, plus
trastuzumab, 4 mg/kg by intravenous infusion every 14 days (loading dose at the first administration 6 mg/kg).

RESPONSE ASSESSMENT

For the purposes of this study, patients should be reevaluated for response every 8 weeks (every 4 weeks for superficial lesions). In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response. Evaluation of response to treatment will be done according to RECIST criteria.

STUDY OBJECTIVES AND SAMPLE SIZE CONSIDERATIONS

Primary endpoint:

- To assess the activity of this regimen in terms of overall clinical benefit, defined as the objective response rate plus the rate of stable disease lasting longer than 24 weeks.

Secondary endpoints:

- To describe the toxicity of the regimen.
- To estimate the time to disease progression and the overall survival.
- To evaluate the changes in levels of circulating endothelial cells, apoptotic endothelial cells and endothelial cell progenitors, during treatment.

Statistical Considerations

An optimal two-stage design will be adopted to test the null hypothesis that $p \leq 0.4$ vs. the alternative that $p \geq 0.6$ with $\alpha=0.05$ e $\beta =0.1$

The expected sample size is 36 with a probability of early termination of 0.73.

The probability of concluding that treatment will be effective when actually it is not is 0.049 (target probability is 0.05). On the other hand if the regimen is actually effective, there is a 0.099 probability of concluding that it is not (the target for this value was 0.100).

After testing the regimen on 25 patients in the first stage, the trial will be terminated if 11 or fewer respond. If the trial goes on to the second stage, a total of 66 patients will be studied. If the total number responding is less than or equal to 32, the regimen will be rejected.

The rates of objective response and clinical benefit will be calculated, with exact confidence intervals.

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APPENDIX A

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A phase II study of metronomic oral chemotherapy with cyclophosphamide plus capecitabine combined with trastuzumab in advanced breast cancer

1. OBJECTIVES

1.1. Primary objectives:

- 1.1.1. To assess the activity of a regimen of metronomic chemotherapy with oral cyclophosphamide plus capecitabine in combination with intravenous trastuzumab in terms of overall clinical benefit, defined as the objective response rate plus the rate of stable disease lasting longer than 24 weeks.

1.2. Secondary objectives:

- 1.2.1. To describe the toxicity of this regimen.
- 1.2.2. To estimate the time to disease progression and the overall survival.
- 1.2.3. To evaluate the changes in levels of circulating endothelial cells, apoptotic endothelial cells and endothelial cell progenitors during treatment.

2. BACKGROUND

2.1 Breast cancer and the angiogenic process

Breast cancer is the most common cancer and the leading cause of cancer death in women in Europe, with estimated 350000 new cases and 130000 deaths recorded in the year 2000 (26.5% of all new cancer cases and 17.5% of all cancer deaths) (1). Although many cytotoxic agents are active in breast cancer, the advanced disease remain incurable and new treatment strategies are urgently needed. Primary tumors and metastases require the formation of new blood vessels in order to grow beyond about 2 mm³ in size (2). Angiogenesis, the formation of new vessels from pre-existing ones, and vasculogenesis, the assembly of vessels from endothelial precursors originating from the bone marrow, are therefore key processes for tumor development and relevant targets for tumor control (3).

Tumor angiogenesis is strictly regulated by a number of stimulatory and inhibitory molecules. The vascular endothelial growth factor (VEGF) family of stimulators is the main player in many tumor types, promoting endothelial cell survival, division, migration, as well as vascular permeability and mobilization of immature bone-marrow-derived endothelial progenitor cells into the peripheral circulation (4). Tumor production of VEGF may be the result of genetic events, including the inactivation of tumor suppressor genes like p53, PTEN or VHL, or the activation of oncogenes, among which src, ras, bcr-abl, EGFR and c-erbB2; it may also be the result of epigenetic events, including environmental factors like hypoxia and altered pH, or exposure to the action of some growth factors, inflammatory cytokines and hormones (4).

Preclinical studies have shown that during breast cancer development angiogenesis accompanies or even precedes the transformation of hyperplasia to malignancy, the balance between metalloproteinases (MMP) and their inhibitors is disrupted in favor of MMP extracellular degradation activity, and the expression of hypoxia-inducible factors (HIF-1 and HIF-2) progressively increases (5). Clinicopathologic studies have highlighted the poor prognostic impact of tumor microvessel density (MVD) and VEGF expression (6), as well as their association with impaired response to chemotherapy and endocrine therapy (7). Invasive breast cancers have been shown to produce a number of angiogenesis stimulators, among which the 121 amino acid isoform of VEGF predominates (8). Tumor progression involves an increase in angiogenic activity with successive contribution of different stimulators, like bFGF (basic fibroblast growth factor), TGFβ-1 transforming growth factor β-1, PLGF (placental growth factor), PD-ECGF (platelet-derived endothelial cell growth factor), pleiotrophin, etc. This could represent a mechanism of resistance to antiangiogenic drugs, and make the control of this process more difficult in more advanced phases of the disease. It also suggests the need for combined inhibition of multiple pathways or the sequential addition of different antiangiogenic agents as strategies for long term tumor control (9).

2.2 Antiangiogenic therapy with trastuzumab

There is compelling evidence from experimental works that inhibiting angiogenesis may induce tumor regression or sometimes even cure. Targeting tumor vasculature, composed of genetically stable endothelial cells, has been regarded as a means to overcome the acquired drug-resistance which characterizes cancer cells, which are genetically unstable and prone to mutations.

Her2/neu is a 185-kilodalton transmembrane receptor tyrosine kinase that belongs to the epidermal growth factor receptor family [10,11,12]. Tumor overexpression of HER2/neu is present in about 30% of patients with breast cancer and is associated with a worse histological grade,

decreased overall survival and altered sensitivity to chemotherapeutic agents [13,14]. Recently, Her2/neu has been implicated in tumor angiogenesis. Experimental studies suggest that neutralizing antibodies against Her2/neu or EGFR result in down-regulation of angiogenesis, through VEGF gene suppression [15]. Data have reported that such interaction occurs *via* abrogation of the increased synthesis of HIF1 α (hypoxia inducible factor-1 α) induced by c-erbB2 activation by ligands (i.r. heregulin) (16). Moreover, an hypoxic-independent mechanisms has been recently advocated in the angiogenetic involvement of HER2-/neu [17].

Trastuzumab (Herceptin®; Genentech, South San Francisco, CA), a recombinant humanized anti-erbB2/HER-2 monoclonal antibody (MoAb) used in erbB2-overexpressing breast carcinoma, has been shown to have antiangiogenic properties [18]. Trastuzumab can induce normalization and regression of the vasculature in an experimental human breast tumor overexpressing HER2 in mice, by modulating the effects of different pro- and anti-angiogenic factors [18]. The combination of trastuzumab with chemotherapeutic agents (paclitaxel, docetaxel) have been shown to increase the efficacy of trastuzumab in reducing angiogenesis in erbB2 overexpressing cells more than either therapy alone both in animal models and clinical studies [19-20].

2.3 Metronomic chemotherapy

Many chemotherapeutic agents have been shown to exert cytotoxic effects not only on tumor cells but also on the endothelial cells of tumor microvasculature. This anti-angiogenic activity seems prominent with the protracted exposure to low doses of chemotherapeutics, compared with their cyclic administration at the maximum tolerated dose [21]. The term 'metronomic' chemotherapy refers to the frequent, even daily, administration of chemotherapeutics at doses significantly below the maximum tolerated dose, with no prolonged drug-free breaks [22]. In vivo preclinical experiments have demonstrated that the metronomic administration of a chemotherapeutic drug can be effective in inhibiting the growth of tumors whose cells have developed resistance against the same drug, and have shown that the apoptosis of vascular endothelial cells precedes that of tumor cells, thus implying an action primarily on the vasculature [23]. Preclinical experiments have also shown that a combination of metronomic chemotherapy with a 'dedicated' anti-angiogenic drug is more active than either therapy alone, inducing remarkable responses and improving survival without additional toxicity [24]. These experimental evidences have prompted the exploration of such strategies in clinical trials. A first experience with metronomic chemotherapy, conducted at the European Institute of Oncology, involved the administration of oral cyclophosphamide 50 mg daily and oral methotrexate 2.5 mg twice daily two days per week (CM regimen). Among 63 evaluable patients previously treated for metastatic breast cancer, this regimen yielded a response rate of 19% and a clinical benefit, defined as either objective response or stable disease longer than 24 weeks, of 32%, in the absence of serious toxicity, with a marked drop in circulating VEGF [25]. In a subsequent study at the same institution, 171 patients with advanced breast cancer, either pre-treated or not with chemotherapy, have been randomized to CM alone or CM + thalidomide. In this less heavily pretreated group of patients, CM alone yielded an objective response rate of 20.9% (95%CI 12.9-31.0%) and an overall clinical benefit of 41.5% (95%CI 34.0-49.3%) [26]. In a previous small series, low-dose, oral cyclophosphamide and methotrexate combined with trastuzumab have been shown to have substantial efficacy in metastatic HER2 positive breast cancer and provided a control of the disease (clinical benefit) in a significant proportion of patients. The observed clinical benefit

(RP plus RC plus SD for ≥ 24 weeks) in all patients and in patients with disease resistant to previous trastuzumab therapy were 46% (95% CI, 24-68%) and 27% (95% CI, 6-61%), respectively [27].

The Dana Farber/Partners CancerCare has conducted a randomized phase II trial comparing the CM metronomic regimen with the same regimen in combination with bevacizumab 10 mg/kg every 2 weeks, in women with advanced breast cancer treated at most with one prior regimen. A planned interim analysis after the first 19 patients per arm has revealed a significant advantage in favor of the combined arm, with 7 versus 3 objective remissions and 6 versus 4 stable diseases [28]. An analogous strategy, with daily oral cyclophosphamide plus bevacizumab, has yielded a 28% objective response rate and 62% stable disease rate in 29 patients with platinum and taxane pretreated ovarian cancer [29].

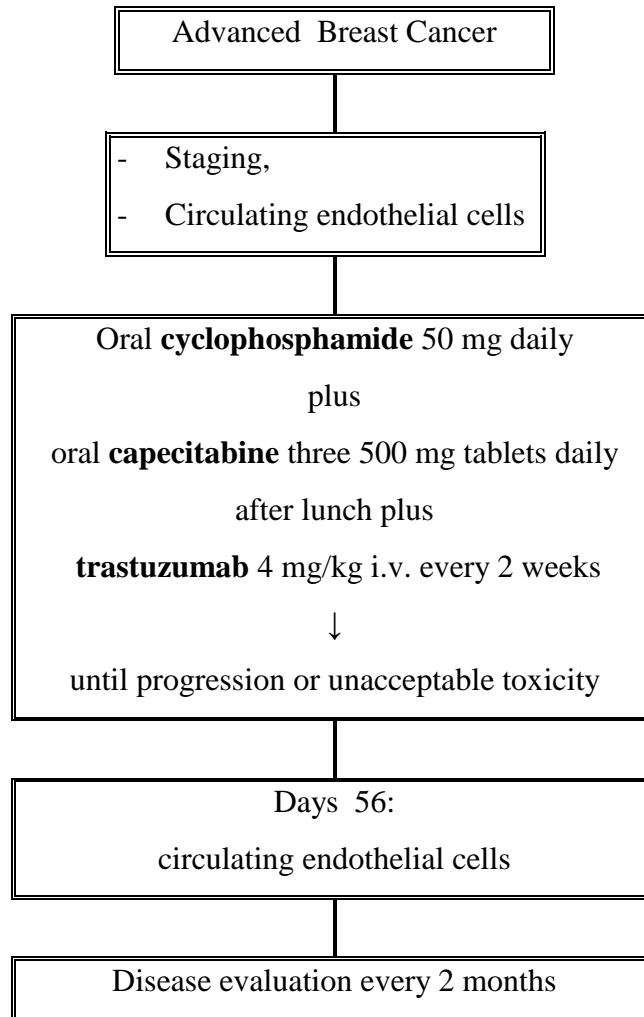
Capecitabine has proved activity in advanced breast cancer. Its daily oral administration mimics a continuous intravenous infusion of fluorouracil, which has antiangiogenic activity in preclinical models. The standard schedule of 1250 mg twice daily for 14 days every 21 days yields objective response rates of about 15-35% as first and second line treatment. Lower doses are better tolerated, and from retrospective evaluations do not seem to compromise efficacy [30]. Limited experience is available with fixed daily doses of capecitabine given continuously, but good tolerability and evidence of clinical activity are reported, as well as the ease of combination with other therapies [31]. For its pharmacokinetic and toxicological features, capecitabine seems particularly suitable for metronomic administration. Other authors have reported good antitumor activity and tolerability with combination regimens including capecitabine and cyclophosphamide [32-33]. Recent data from a phase II study reported the high rate of clinical benefit associated with mild and manageable toxicity with the daily oral administration of cyclophosphamide, 50 mg daily, and capecitabine, 500 mg thrice daily, in combination with bevacizumab. An overall response rate of 48% and a clinical benefit rate of 68% were reported [34]

2.4 *Rationale of the clinical and correlative biological study*

It has not been defined which is the best way to administer metronomic chemotherapy. Biological considerations, like the relentless growth of tumor microvasculature, and pharmacokinetic considerations concerning oral agents, suggest that a frequent administration of the chemotherapeutic agent, daily or even more times a day, should be preferred, mimicking more strictly a continuous infusion of the drug. Because bone marrow suppression is a potent proangiogenic stimulus, inducing the release of hematopoietic stem cells including endothelial progenitor cells, it has been suggested that the dose to be used in metronomic therapy should not cause bone marrow perturbation [35]. Based on these considerations, the new regimen we planned involves the administration of a low total daily dose of capecitabine (1500 mg overall) in three divided doses.

The clinical development of antiangiogenic therapies would be greatly expedited by the identification of surrogate markers of activity. Recently, the number of circulating endothelial cells and endothelial precursors has been proposed as a promising surrogate marker for angiogenesis, and validated in preclinical experiments showing its high correlation with tumor angiogenesis [36]. Based on these data, the activity of the proposed regimen in this trial will be evaluated also by measuring circulating endothelial cells and precursors.

SCHEMA



3. PATIENT SELECTION

3.1 Eligibility Criteria

- 3.1.1 Pre- or post-menopausal women with histologically proven, locally advanced (inoperable) or metastatic breast carcinoma.
- 3.1.1 Measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan. See section 9.2 for the evaluation of measurable disease.
- 3.1.2 No previous lines of chemotherapy for advanced disease (primary and/or adjuvant chemotherapy are allowed, as well as any prior endocrine treatment; prior primary and/or adjuvant trastuzumab therapy are allowed, if ended ≥ 6 months before). At least 4 weeks must have elapsed since prior chemotherapy or radiation therapy, 6 weeks if the last regimen included mitomycin C).
- 3.1.5 Age ≥ 18 years and < 80 years.
- 3.1.6 Life expectancy of greater than 6 months.
- 3.1.7 ECOG performance status ≤ 2 (Karnofsky $\geq 60\%$; see Appendix A).
- 3.1.8 Patients must have normal organ and marrow function as defined below:
- | | |
|-----------------------------|--|
| - leukocytes | $\geq 3,000/\mu\text{L}$ |
| - absolute neutrophil count | $\geq 1,500/\mu\text{L}$ |
| - platelets | $\geq 100,000/\mu\text{L}$ |
| - total bilirubin | within normal institutional limits |
| - AST(SGOT)/ALT(SGPT) | ≤ 2 X institutional upper limit of normal |
| - creatinine | within normal institutional limits |
- OR
- | | |
|------------------------|---|
| - creatinine clearance | ≥ 60 mL/min/1.73 m ² for patients with creatinine levels above institutional normal |
|------------------------|---|
- 3.1.9 Geographically accessible for follow up.
- 3.1.10 Women of child-bearing potential must agree to use adequate contraception prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
- 3.1.11 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.2 Previous or concomitant other malignancy except basal or squamous cell carcinoma of the skin or adequately treated in situ carcinoma of the cervix.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to trastuzumab or other agents used in the study.

3.2.4 Serious cardiac illness or medical conditions including but not confined to:

- History of documented congestive heart failure (CHF) or systolic dysfunction (LVEF <50%)
- High risk uncontrolled arrhythmias (ventricular tachycardia, high grade AV-block, supraventricular arrhythmias which are not adequately rate-controlled);
- Angina pectoris requiring antianginal medication
- Clinically significant valvular heart disease
- Evidence of transmural infarction on ECG
- Poorly controlled hypertension (systolic >180 mmHg or diastolic > 100 mmHg)

3.2.3 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, renal failure, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.4 Pregnant women are excluded from this study because of the potential for teratogenic or abortifacient effects of trastuzumab and other agents included in this trial. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother, breastfeeding must be discontinued.

4. HER2 SCREENING FOR ELEGIBILITY

Patient eligibility must include HER2 overexpression as defined by score of 3+ (>30% of invasive tumor cells) by IHC or HER2 gene amplification (> 6 HER2 gene copies per nucleus) or a ratio (HER2 gene copies to chromosome 17 signals) of > 2.2 determined by FISH/CISH alone, or in conjunction with a 2+ IHC score or + in 30% or less neoplastic cells on the invasive component of the tumor. Central confirmation (Pathology Lab at the European Institute of Oncology, Milan) of a positive HER2 status is mandatory prior to enrolment to the study (see Appendix)

Also hormone (ER/PgR) receptor status will be tested centrally on tissue specimens of patients prior to enrolment.

All these test are to be done in formalin-fixed paraffin-embedded blocks (FFPE)

5. TREATMENT PLAN

5.1 Treatment schedule

Cyclophosphamide, one 50 mg tablet daily (at 9 AM), plus

Capecitabine, three 500 mg tablets daily after lunch, plus

Trastuzumab, 4 mg/kg by intravenous infusion every 14 days (loading dose at first administration 6 mg/kg)

5.2 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for trastuzumab, cyclophosphamide, and capecitabine are described in Section 7. Appropriate dose modifications for trastuzumab and chemotherapeutic agents are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.2.1 *Trastuzumab*

Trastuzumab (HERCEPTIN®) (Genentech/Roche Genentech, South San Francisco, CA.) will be administered at the dose of 4 mg/kg by intravenous infusion over 60 minutes, every 14 days. A loading dose of 6 mg/kg by intravenous infusion over 90 minutes is required at first administration.

Each vial of trastuzumab 150 mg is reconstituted with 7.2 mL of Sterile water for Injection. This formulation does not contain a preservative and is suitable for single use only. Appropriate aseptic techniques should be used. Trastuzumab should be carefully handled during reconstitution. The following instructions have to followed:

- 1) Using a sterile syringe, slowly inject the sterile water for injections in the vial containing the lyophilized trastuzumab, directing the stream into the lyophilized cake.

2) Swirl vial gently to aid reconstitution. Do not shake.

The reconstituted solution contains 21mg/ml of trastuzumab, at pH of approximately 6.0, and the appropriate calculated volume will be added to 250 mL of 0.9% Sodium Chloride Injection. Determine the volume of the solution required based on loading dose of 4 mg trastuzumab /Kg body weight (or the loading dose of 6 mg/ trastuzumab /Kg body weight).

Volume (in ml) to be added = $\frac{\text{body weight (kg)} \times \text{dose (4 mg/kg or 6 mg/Kg)}}{21 \text{ mg/mL}}$

If diluted aseptically, it may be stored for a maximum of 24 hours from reconstitution (do not store above 30°C).

Trastuzumab will be administered at the dose of 4 mg/kg by intravenous infusion every 14 days (loading dose at first administration 6 mg/kg over 90 minutes) over 60 minutes. Premedication with paracetamol is required.

4.1.2 Cyclophosphamide

Cyclophosphamide (ENDOXAN ®) (Asta Medica Italia) is available as 50 mg tablets and is administered at the dose of 50 mg daily (one tablet daily) without interruptions, approximately at 9 AM, with water.

4.1.3 Capecitabine

Capecitabine (XELODA ®) (Roche Italia S.p.A) is available as 500 mg tablets and is administered at the fixed dose of 1500 mg daily (three 500 mg tablets) without interruptions, after lunch, with water.

4.2 **Supportive Care Guidelines**

Patients may receive all commonly used supportive care measures in order to control side-effects of treatment.

4.3 **Duration of Therapy**

In the absence of treatment delays or temporary interruptions due to adverse events, treatment may continue until one of the following criteria applies:

- X Disease progression,
- X Intercurrent illness that prevents further administration of treatment,
- X Unacceptable adverse events(s),
- X Patient decides to withdraw from the study, or
- X General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

6. DOSING DELAYS/DOSE MODIFICATIONS

Adverse events will be classified according to the NCI Common Terminology Criteria For Adverse Events, version 3 (CTCAE) [37] (<http://ctep.cancer.gov/forms/CTCAEv3.pdf>).

6.1 Trastuzumab

There are no recommended dose reductions for the use of trastuzumab. If needed, trastuzumab should be either discontinued or temporarily suspended as described below.

6.1.1 Action to be taken in case of trastuzumab related adverse events

Adverse events	Action
Non-hematological grade 1-2	Continue T therapy
Non-hematological grade 3-4	Hold T until recovery to grade \leq grade2
Non-hematological grade 3-4 upon rechallenge with T	Discontinue T
Cardiac (asymptomatic drop in LVEF or symptomatic congestive heart failure)	Hold T , continued or resumed according to figure1 for NYHA Class I or II CHF . Discontinue T if NYHA Class I or II CHF .
Hematological	T dose should not be held

6.1.2 Infusion associated symptoms with trastuzumab therapy

Patients with pulmonary disease or pre-existing respiratory compromise may be at increased risk from serious symptoms. Patients who experience a life-threatening infusion reaction on the first dose (tachypnea, bronchospasm, hypotension, hypoxia), should be withdrawn from study medications. Patients who experience severe or moderate infusion symptoms may be managed by:

- slowing or stopping the trastuzumab infusion
- supportive care with oxygen, beta agonist, antihistamines, corticosteroids for example.

Patients that experience mild or moderate infusion symptoms may be treated with antipyretic and antihistamines.

Patients who experienced mild, moderate or severe infusion reactions on the first dose may be retreated with trastuzumab. Premedication with corticosteroids, antihistamines, and antipyretics may be used before subsequent trastuzumab infusion.

6.1.3 Cardiac monitoring

All patients must have a LVEF measurement of at least 50% by echocardiography or MUGA scan, a maximum of 30 days prior to randomization. Subsequent scheduled LVEF assessments must be performed every 3 months during treatment and every 6 months during follow-up.

6.2 Chemotherapeutic Agent(s)

To achieve a 50% dose reduction, give cyclophosphamide one 50 mg tablet every other day, and capecitabine one 500 mg tablet once daily (after dinner, on days taking cyclophosphamide if both reduced) alternated with one 500 mg tablet twice daily (after breakfast and after dinner).

6.2.1 Hematological toxicity

Complete blood count must be repeated every two weeks during the first month of treatment, and monthly thereafter.

Cyclophosphamide and capecitabine will be administered at full dose if leukocytes are equal to or greater than $3000/\text{mm}^3$ and platelets are equal to or greater than $100.000/\text{mm}^3$. If leukocytes and/or platelets are lower than these values, treatment will be administered according to the following criteria:

Platelets	Leukocytes		
	$\geq 3.0 \times 10^9/l$	$2.0 - 3.0 \times 10^9/l$	$< 2.0 \times 10^9/l$
$\geq 100 \times 10^9/l$	100%	50%	0%
$75 - 99 \times 10^9/l$	50%	50%	0%
$< 75 \times 10^9/l$	0%	0%	0%

Any platelets toxicity of grade ≥ 2 and leukocyte toxicity of grade ≥ 3 should be managed with temporary interruption of all chemotherapeutic agents until recovery at least to a grade 1, when treatment may be resumed with a 50% dose reduction. Re-escalation of drug doses should only be attempted if close monitoring is possible.

6.2.2 Renal dysfunction

Cyclophosphamide and capecitabine should be administered only in presence of normal renal function (serum creatinine within normal limits and/or creatinine clearance $\geq 60 \text{ mL/min/1.73 m}^2$). Serum creatinine should be monitored monthly.

6.2.3 Cystitis

All patients should be instructed as to the importance of high fluid intake during cyclophosphamide therapy. If grade ≥ 2 cystitis occur despite hydration, cyclophosphamide treatment should be stopped until recovery and restarted at 50% of dose of cyclophosphamide.

6.2.4 Gastrointestinal toxicity

In case of grade 2 gastrointestinal toxicity (anorexia, nausea, vomiting, stomatitis, diarrhea, etc.) the dose of all chemotherapeutic agents should be reduced by 50% and, if

there is no improvement at least to grade 1 toxicity, these drugs should be temporarily interrupted. In case of grade ≥ 3 gastrointestinal toxicity, all chemotherapeutic agents should be stopped. Treatment may be resumed after recovery at least to grade 1 toxicity, starting with 50% dosage, with subsequent re-escalation to full dosage if tolerated.

6.2.5 Hand-foot syndrome

In case of grade 2 hand-foot syndrome, therapy with pyridoxine (Benadon®), one 300 mg tablet daily will be prescribed and capecitabine will be continued. In case of no benefit, as well as in case of grade 3 toxicity, capecitabine will be temporarily interrupted and resumed at half dose only after recovery to at least grade 1 toxicity, with subsequent re-escalation (in association with pyridoxine therapy) if tolerated.

6.2.6 Other toxicity

If deemed necessary, dosage may be reduced for other toxicities.

7. AGENT FORMULATION AND PROCUREMENT

7.1 Trastuzumab

Each vial of trastuzumab 150 mg is reconstituted with 7.2 mL of Sterile water for Injection. This formulation does not contain a preservative and is suitable for single use only. Appropriate aseptic techniques should be used. Trastuzumab should be carefully handled during reconstitution. The following instructions have to be followed:

- 3) Using a sterile syringe, slowly inject the sterile water for injections in the vial containing the lyophilized trastuzumab, directing the stream into the lyophilized cake.
- 4) Swirl vial gently to aid reconstitution. Do not shake.

The reconstituted solution contains 21 mg/ml of trastuzumab, at pH of approximately 6.0, and the appropriate calculated volume will be added to 250 mL of 0.9% Sodium Chloride Injection. Determine the volume of the solution required based on loading dose of 4 mg trastuzumab /Kg body weight (or the loading dose of 6 mg/ trastuzumab /Kg body weight).

Volume (in ml) to be added = $\frac{\text{body weight (kg)} \times \text{dose (4 mg/kg or 6 mg/Kg)}}{21 \text{ mg/mL}}$

If diluted aseptically, it may be stored for a maximum of 24 hours from reconstitution (do not store above 30°C).

7.2 Chemotherapeutic Agents

7.2.1 Cyclophosphamide

Cyclophosphamide is a cell cycle nonspecific alkylating agent. It is activated by the hepatic microsomal enzymes to 4-hydroxycyclophosphamide which is in equilibrium with its tautomer aldophosphamide, which eliminates acrolein to produce phosphoramide mustard, the active alkylating agent. The mechanism of action involves the cross-linking of DNA in tumor cells. Oral bioavailability is 100%. Metabolized in the liver, cyclophosphamide is eliminated primarily as metabolites in the urine; half-life is 4-6.5 hours.

Main side effects are:

- Hematological: leucopenia, thrombocytopenia
- Gastrointestinal: nausea and vomiting, anorexia, mild stomatitis and diarrhea
- Hepatotoxicity
- Renal/genitourinary: hemorrhagic or non hemorrhagic cystitis, hyponatremia, hyperkalemia, hyperuricemia, inappropriate antidiuretic syndrome (SIADH)
- Pulmonary
- Cardiac (with high doses): congestive heart failure, myocarditis, pericarditis
- Dermatological: alopecia, hyperpigmentation of nails and skin, phlebitis at the injection site, nonspecific dermatitis
- Hypersensitive/allergic/anaphylactic reactions (rare)
- Reproductive: amenorrhea, oligospermia or azoospermia,

- Secondary neoplasias: is carcinogenic and mutagenic

7.2.2 Capecitabine

Capecitabine is an oral prodrug, which is converted to its active metabolite fluorouracil by thymidine phosphorilase, an enzyme present at higher levels in several tumor types compared to normal tissues. Daily administration mimics continuous infusion of fluorouracil. Bioavailability is about 100%, with peak at 1.5-2 hours, and terminal half-life of the active metabolite of about one hour.

Toxicity (highly increased in patients with DPD deficiency) includes:

- Hematological: leucopenia, anemia
- Gastrointestinal: diarrhea, nausea, vomiting, stomatitis, anorexia, abdominal pain, constipation, dyspepsia
- General: asthenia, weakness, hyperpyrexia,
- Hepatic: hyperbilirubinemia
- Cardiac: myocardial ischemia-infarction, arrhythmias
- Neurological: dizziness, headache, lethargy
- Dermatological: “hand-foot syndrome” depicted by painful, erythematous desquamation and fissures of palms and soles; rash, alopecia, hyperpigmentation of skin, pruritic maculopapular rash on the extremities (sun exposure tends to initiate or increase skin reactions;
- Hypersensitivity reactions
- Reproductive: the drug has mutagenic and teratogenic properties
- Miscellaneous: photophobia, conjunctivitis, blurred vision, increased lacrimation

8. CORRELATIVE/SPECIAL STUDIES

8.1 Evaluation of circulating markers of angiogenic activity:

Circulating parameters of angiogenesis will be measured:

- at baseline
- at week 8
- at the documentation of disease progression

8.1.1. circulating endothelial cells (CECs), circulating endothelial precursors (CEPs), apoptotic CECs

Sample Collection.

Twenty-six millilitres of native blood will be collected and divided into 6 tubes: two plain tubes (five millilitres per tube), one containing citrate solution (five millilitres per tube), and three containing EDTA (3.5 millilitres per tube), all of them labelled with protocol number, patient identification number, patient's initials and date of collection. A Transporting Form must be filled out and submitted with each set of samples. The tubes must be treated as follows:

1. the two plain tubes should be allowed to clot for two hours at room temperature, centrifuged at 3000 rpm for 10 min, and then separated
2. the EDTA and citrated tubes should be immediately centrifuged at 3000 rpm for 10 min and then separated
3. the serum and the plasma must be separated in 500 ml aliquots (at least 6 for serum and 4 for plasma), and put into cryogenic tubes on which the following information must be reported:
 - a. protocol number
 - b. patient's identification number (randomization number) and the patient's initials
 - c. type of sample: S= serum or P= citrated plasma
 - d. date of collection

The cryogenic vials should be put into the appropriate cryogenic box, and a precise box map must be completed. Each box must be identified by a progressive letter, and the same letter must be reported on the Box Map form. The cryogenic box must be stored in a - 80 °C freeze.

Sample evaluation:

CECs and CEPs evaluation will be performed by Flow Cytometry. Monoclonal antibodies including anti-CD45 to exclude hematopoietic cells, anti-CD31, -CD133, -P1H12, the apoptosis marker 7-aminoactinomycin D, and appropriate analysis gates will be used to enumerate viable and apoptotic CECs and CEPs. Cell suspensions will be evaluated after red cell lysis by a FACSCalibur equipped with a second red-diode laser (BD Biosciences, San Jose, CA). After acquisition of at least 100,000 cells/blood sample, analyses will be considered as informative when adequate numbers of events (*i.e.*, >100) will be collected in the CECs enumeration gates. CECs are defined as negative for hematopoietic marker CD45, positive for endothelial markers P1H12 and CD31, and negative for the progenitor marker CD133. CEPs are depicted by expression of CD133.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done within 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Off Study
<i>Trastuzumab</i>		A		A		A		A		A		A		
<i>Metronomic chemotherapy</i>		B	B	B	B	B	B	B	B	B	B	B	B	
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X-----X												
Physical exam	X	X		X		X		X		X		X		X
Vital signs	X	X		X		X		X		X		X		X
Height	X													
Weight	X	X		X		X		X		X		X		X
Performance status	X	X		X		X		X		X		X		X
CBC w/diff, plts	X			X		X		X		X		X		X
Serum chemistry ^a	X			X		X		X		X		X		X
EKG (as indicated)	X													
Adverse event evaluation		X-----X												X
Tumor measurements	X	Measurement of superficial lesions is repeated every <u>4</u> weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X
Radiologic evaluation	X	Radiologic measurements should be performed every <u>8</u> weeks.												X
B-HCG	X ^b													

A: *Trastuzumab*: 4 mg/kg i.v. every 14 days
 B: Metronomic chemotherapy:
 ▪ cyclofosamide 50 mg daily + capecitabine 500 mg thrice daily
 a: BUN, creatinine, Na, K, Cl, Ca, glucose, γ GT, AST, ALT, ALP, LDH, total and fractionated bilirubin, PT, PTT, fibrinogen, fibrin split products (+ antithrombin III, total protein and albumin at baseline and every 8 weeks)
 b: Serum pregnancy test (women of childbearing potential).
 c: endothelial cells, endothelial precursors, endothelial apoptotic cells.

Baseline evaluations

- Medical history
- Concurrent medications
- Physical examination
- Vital signs: arterial pressure, pulse rate
- Height and weight
- Performance status
- CBC
- Serum chemistry: urea nitrogen, creatinine, sodium, potassium, chloride, calcium, glucose, total protein, albumin, γ -glutamyl-transpeptidase, aspartate and alanine aminotransferases, alkaline phosphatase, lactate dehydrogenase, total and fractionated bilirubin
- Serum pregnancy test (women of childbearing potential)
- Tumor markers: CEA and/or CA15/3
- Electrocardiogram and cardiologic consultation
- Chest X-rays / CT scan of the thorax
- Abdomen US or CT
- Circulating endothelial cells, endothelial precursors, endothelial apoptotic cells
- Other exams necessary for tumor assessment
- Other radiological assessments as clinically indicated

Fortnightly evaluations

- Assessment of toxic effects
- Physical examination
- Performance status
- Vital signs: arterial pressure, pulse rate
- CBC
- Serum chemistry: urea nitrogen, creatinine, sodium, potassium, chloride, calcium, glucose, γ -glutamyl-transpeptidase, aspartate and alanine aminotransferases, alkaline phosphatase, lactate dehydrogenase, total and fractionated bilirubin,

Evaluations every 8 weeks

- Serum chemistry (further than fortnightly exams): total protein, albumin
- Tumor markers: CEA and/or CA15/3
- Tumor assessment by the same exams used at baseline
- Other radiological assessment as clinically indicated
- Circulating endothelial cells, endothelial precursors, endothelial apoptotic cells

Evaluation at the end of the study

- Assessment of toxic effects
- Physical examination
- Performance status
- Vital signs: arterial pressure, pulse rate
- CBC
- Serum chemistry: urea nitrogen, creatinine, sodium, potassium, chloride, calcium, glucose, γ -glutamyl-transpeptidase, aspartate and alanine aminotransferases, alkaline phosphatase, lactate dehydrogenase, total and fractionated bilirubin,
- Tumor markers: CEA and/or CA15/3
- Tumor assessment
- Other radiological assessment as clinically indicated
- Circulating endothelial cells, endothelial precursors, endothelial apoptotic cells

10. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be reevaluated for response every 8 weeks (every 4 weeks for superficial lesions). In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

10.1. Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [38 *JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term “evaluable” in reference to measurability will not be used because it does not provide additional meaning or accuracy.

10.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques (CT, MRI, x-ray) or as ≥ 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

10.1.2 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

10.1.3 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 baseline. 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 baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

10.1.4 Non-target lesions

All other lesions (or sites of disease) should be identified as **non-target lesions**

and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

10.2 **Guidelines for Evaluation of Measurable Disease**

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Note: Tumor lesions that are situated in a previously irradiated area may be considered measurable only in case of superficial lesions involving soft tissues, that appeared or grew after the end of radiation treatment. Bone or visceral lesions treated with radiotherapy may not be considered measurable.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

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

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI. These techniques 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 tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor 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.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor

response should be restricted to validation purposes in reference centers. However, such techniques can be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10.3 **Response Criteria**

10.3.1 **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 longest diameter (LD) of target lesions, taking as reference the baseline 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

10.3.2 **Evaluation of non-target lesions**

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s)

and/or maintenance of tumor 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

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 at a later time by the review panel (or study chair).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

10.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1).

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

Note:

- X Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration.” Every effort should be made to document the objective progression, even after discontinuation of treatment.
- X In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

9.10.4 **Confirmatory Measurement/Duration of Response**

10.4.1 **Confirmation**

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 8 weeks (see section 9.3.3).

10.4.2 **Duration of overall response**

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference

for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

10.4.3 **Duration of Stable Disease**

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

10.5 **Progression-Free Survival**

PFS is defined as the duration of time from start of treatment to time of progression.

10.6 **Response Review**

For trials where the response rate is the primary endpoint, it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

11 STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

This is a single arm phase II study, whose main objective is to assess the activity of a new regimen of metronomic chemotherapy combined with Trastuzumab in terms of overall clinical benefit, defined as the objective response rate plus the rate of stable disease lasting longer than 24 weeks. Secondary endpoints are to describe the toxicity and to estimate the time to disease progression and the overall survival, as well as to evaluate the impact of treatment on tumor angiogenesis, assessing the changes in levels of circulating endothelial cells.

11.2 Sample Size/Accrual Rate

An optimal two-stage design will be adopted to test the null hypothesis that $p \leq 0.4$ vs. the alternative that $p \geq 0.6$ with $\alpha=0.05$ e $\beta=0.1$

The expected sample size is 36 with a probability of early termination of 0.73.

The probability of concluding that treatment will be effective when actually it is not is 0.049 (target probability is 0.05). On the other hand if the regimen is actually effective, there is a 0.099 probability of concluding that it is not (the target for this value was 0.100).

After testing the regimen on 25 patients in the first stage, the trial will be terminated if 11 or fewer respond. If the trial goes on to the second stage, a total of 66 patients will be studied. If the total number responding is less than or equal to 32, the regimen will be rejected.

The rates of objective response and clinical benefit will be calculated, with exact confidence intervals.

11.3 Analysis of Secondary Endpoints

Analyses of secondary endpoints will be exploratory in nature. Time to events will be estimated by the product limit method. For each time point where biological assessments are planned, the 95% confidence interval for the percent change from baseline will be presented for each circulating angiogenic biomarker. The Wilcoxon signed rank test will be used to compare the levels of circulating angiogenic biomarkers and assessed on day 56 with baseline levels.

11.4 Evaluation of toxicity.

All patients will be evaluable for toxicity from the time of their first treatment with *trastuzumab*

11.5 Evaluation of response.

All patients included in the study will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4-9 will be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration will not result in exclusion from the analysis of the response rate.

12 REGULATORY AND REPORTING REQUIREMENTS

12.1 Case report forms and schedule for completion

Data will be reported on the Medical Oncology Division forms.

Case report forms must be completed according to the following schedule

A. Before the treatment starts:

- the patient must be registered at the Data Management Center
- the following set of forms has to be returned to the Data Management Center:
 - the registration check-list
 - the on-study form
 - a laboratory form, including the last laboratory values obtained before starting treatment
 - the initial measurement form (or picture of the lesion)
 - the concomitant medications form listing all medications assumed by the patient
 - a copy of the original pathology report, including the diagnosis of cancer

The optimal way to work is to complete the registration check-list and, if possible, the above set of forms first, and to register the patient as soon as data are complete. The date of registration and patient sequential identification number are then completed on the check-list, and the whole set can be sent to the Data Center.

B. During therapy:

- At the end of the 1st and 2nd course of treatment, and after each patient visit thereafter, a set of forms has to be sent to the Data Center, including:
 - a treatment form
 - an adverse event form
 - one or several laboratory forms including all laboratory data obtained since the last form submission. Photograph documentation can be associated to forms.

C. After each evaluation of the disease

- a follow-up measurement form has to be returned to the Data Center after each evaluation of the disease

D. As soon as the investigator has decided to stop the treatment

- an end of protocol treatment form (off study form) has to be returned to the Data Center
- the concomitant medications form listing all concomitant medications received during therapy

E. After treatment discontinuation

- A follow-up form has to be returned to the Data Center after each follow-up visit
If the patient is responding, or presents stable disease, the disease will continue to be evaluated according to the schedule defined in this protocol until disease progression, or start of another anticancer treatment.
- Follow-up measurement forms have to be sent to the Data Center after each evaluation of the disease.

F. Upon patient death

- A death report has to be sent to the Data Center

G. Upon occurrence of a Serious Adverse Event

All serious adverse events occurring during the treatment period and within 30 days after the end of the last treatment must be reported to the Data Center.

- A serious adverse event form must be completed and returned to the Data Center within 10 calendar days of the initial observation of the event.

All forms must be dated and signed by the responsible investigator or one of his/her authorized staff members.

12.2 Data flow

The case report forms must be completed and signed by the investigator or one of his/her authorized staff members as soon as the requested information is available, according to the above described schedule.

The list of staff members authorized to sign case report forms (with a sample of their signature) must be sent to the Data Center by the responsible investigators before the start of the study.

In all cases, it remains the responsibility of the investigator to check that original case report forms are sent to the Data Center and that they are completely and correctly filled out.

The original copy must be immediately returned to the Data Center and the investigator must keep a copy.

The Data Center will perform extensive consistency checks on the CRFs and issue Query Forms in case of inconsistent data. Those Query Forms must be immediately answered and signed by the investigator (or an authorized staff member). The original must be returned to the Data Center and a copy must be appended to the investigator's copy of the CRFs.

If an investigator (or an authorized staff member) needs to modify a CRF after the original copy has been returned to the Data Center, he/she should notify the Data Center in writing (and sign the notification) and append a copy of the notification to his own copy of the CRFs.

The investigator's copy of the CRFs may not be modified unless modifications are reported on a Query Form (or a written and signed notification) and the Query Form (or notification) reference is indicated on the CRF.

13. ADVERSE EVENT REPORTING

13.1 Definitions

An **Adverse Event (AE)** is defined as any untoward medical occurrence or experience in a patient or clinical investigation subject which occurs following the administration of the trial medication regardless of the dose or causal relationship. This can include any unfavorable and unintended signs (such as rash or enlarged liver), or symptoms (such as nausea or chest pain), an abnormal laboratory finding (including blood tests, x-rays or scans) or a disease temporarily associated with the use of the study medication. The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 2.0 will be utilized for adverse event reporting.

An **Adverse Drug Reaction (ADR)** is defined as any response to a medical product, that is noxious and/or unexpected, related to any dose.

Responses to a medicinal product (used in the above definitions) means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

A **Serious Adverse Event (SAE)** is defined as any undesirable experience occurring to a patient, whether or not considered related to the protocol treatment. A serious adverse event (SAE) which is considered related to the protocol treatment is defined as a **Serious Adverse Drug Reaction (SADR)**. Adverse events and adverse drug reactions which are considered as serious are those which result in:

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- persistent or significant disability/incapacity
- a congenital anomaly/birth defect
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above).

The expected adverse events specific for sodium valproate are described in details in Section 2.1.3 and are summarized in Section 6 (Agent Formulation and Procurement).

13.2 Reporting procedure

13.2.1 Non-serious adverse events and non-serious adverse drug reactions

All Adverse Events (AE) and Adverse Drug Reactions (ADR) occurring during the treatment period and within 30 days after the last protocol treatment administration, must be recorded on the toxicity forms.

The investigator will decide if those events are related to the medicinal product (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the toxicity forms. AE definitely not drug related (i.e. reported as unrelated) will not be considered as adverse drug reactions in toxicity analyses, but reported separately.

The assessment of causality is made by the investigator using the following definitions:

13.2.2 Relationship Description

UNRELATED There is no evidence of any causal relationship.

UNLIKELY There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).

POSSIBLE There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).

PROBABLE There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

DEFINITELY There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

NOT ASSESSABLE There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

13.2.3 Serious adverse events or serious adverse drug reactions

All Serious Adverse Events (SAE), related or not to the study treatment, occurring during the treatment period and within 30 days after the last protocol treatment administration, must be reported to the Data Center.

Any late Serious Adverse Drug Reaction (SADR), occurring after this 30-day period also must be reported to the Data Center.

This must be done within 24 hours of the initial observation of the event. Details should be documented on the specified Serious Adverse Event Form.

14. ETHICAL CONSIDERATIONS

14.1 Patient protection

The responsible investigator will ensure that this study is conducted in agreement with the Declaration of Helsinki.

The protocol has been written, and the study will be conducted, according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: <http://www.ifpma.org/pdfifpma/e6.pdf>).

The protocol will be approved by the Local Ethics Committee.

14.2 Subject identification

The name of the patient will not be asked for nor recorded at the Data Center. A sequential identification number will be automatically attributed to each patient registered in the trial. This number will identify the patient and must be included on all case report forms. In order to avoid identification errors, patients initials (maximum of 4 letters), date of birth and local chart number (if available) will also be reported on the case report forms.

14.3 Informed consent

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which they will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. The patient informed consent statement is given at the end of the protocol.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered at the Data Center. The informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

15. ADMINISTRATIVE RESPONSIBILITIES

15.1 The Study Coordinator

The Chairman of the Study will be responsible for writing the protocol, reviewing all case report forms and documenting his/her review on evaluation forms, the contents of the reports, and for publishing the study results. He will also generally be responsible for answering all clinical questions concerning eligibility, treatment, and the evaluation of the patients.

15.2 Trial insurance

The insurance program covers all patients entered on behalf of GOIM in GOIM studies except patients from countries not included in European Community.

15.3 Forms

Forms are provided in allegato.

16. PROPERTY OF DATA AND PUBLICATION POLICY

Property of data is GOIM (Gruppo Oncologico Italia Meridionale).

The main results of the clinical trial will be published in a peer-reviewed scientific journal.

The final publication will be written by one of the Study Chairman on the basis of the final analysis performed by the Ospedale Perrino Statistical Center, Brindisi.

Co-authors will be principal investigators of the Study who participate in the design and drawing up of the research project, the Pathology Study Chair, a representative of the Ospedale Perrino and a representative of the Ospedale Perrino Data Management.

All publications, abstract or presentations including data related to the present trial will be submitted for review to the Chair of the Study prior to submission.

Trials results will not be released before data maturity has been reached for the primary endpoint of the trial.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

