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Coalición Iberoamericana de Investigación en Oncología Mamaria

COALICIÓN IBEROAMERICANA DE INVESTIGACIÓN EN ONCOLOGÍA MAMARIA (CIBOMA) (*IBEROAMERICAN COALITION FOR BREAST ONCOLOGY RESEARCH)*
CIBOMA/2004-01

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER

CIBOMA/2004-01
Chemotherapy vs. Observation

SPONSOR: **CIBOMA (*IBEROAMERICAN COALITION FOR BREAST ONCOLOGY RESEARCH)***

AVENIDA DE LOS PIRINEOS 7, OFICINA 1-14
28703 SAN SEBASTIÁN DE LOS REYES (MADRID)
SPAIN

EudraCT Nº: 2005-002838-36

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Final version (3): June 10, 2005 / Amendment No. 1: June 29, 2007
Amendment No. 2: September 16, 2009

clinical study.

1.- STUDY SUMMARY

Type of request	Clinical trial with a pharmaceutical product in a new indication.
Sponsor details	Coalición Iberoamericana de Investigación en Oncología Mamaria – *Iberoamerican Coalition for Investigation in Breast Oncology* (CIBOMA) Avenida de los Pirineos 7, oficina 1-14 28703 San Sebastián de los Reyes (Madrid) Spain Tel.: + 34 916592870 Fax: +34 916510406 E-mail: ciboma@ciboma.org Web page: www.ciboma.org
Study title	"Phase IV.III, multicenter, open, randomized treatment study to evaluate the efficacy of maintenance therapy with capecitabine (X) after standard (neo-) and/or adjuvant chemotherapy in patients with operated, hormone receptor and HER2neu negative breast cancer".
Protocol Code	CIBOMA/2004-01
Principal investigators	Dr. Ana Lluch, Dr. Ruiz Borrego, Dr. Calvo (Spain), Dr. Laura Torrecillas (Mexico), Dr. Carlos H. Barrios (Brazil) (See Appendix 7).
Centers in which trial will be carried out	University Hospital Clinic, Valencia; University Hospital Virgen del Rocío, Juan Canalejo Hospital Complex (Spain). Specialist Medical Center 20 de Noviembre (Mexico). Hospital Sao Lucas-PUC (Brazil) (See appendix 7).
Clinical Research Ethics Committees which have approved the study	Committees corresponding to the participating centers (see Appendix 7). **Spain:** The Clinical Research Ethics Committee (CREC) of the University Hospital Clinic, Valencia will act as reference CREC for the single approval of the protocol for all centers in Spain.
Good Clinical Practice (GCP) Study Monitors	Yolanda Amigo: yamigo@ciboma.org Victoria Ruiz: vruiz@ciboma.org Nuria Caparroso: ncaparroso@ciboma.org Beatriz Pardo: bpardo@ciboma.org Lourdes del Burgo: lburgo@ciboma.org Patricia Jou: pjou@ciboma.org Anabel García: agarcia@ciboma.org Gema Sanz: gsanz@ciboma.org Sara Perales: sperales@ciboma.org Cristina del Monte: cmonte@ciboma.org
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Study Coordination	Andrés Hernando CIBOMA Avenida de los Pirineos 7, oficina 1-14

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Administrative Coordinators	Elena Gutiérrez and Mª Ángeles Jimeno CIBOMA Avenida de los Pirineos 7, oficina 1-14 28703 San Sebastián de los Reyes (Madrid) Spain Tel: +34916592870 Fax:+34916510406 E-mail: egutierrez@ciboma.org / majimeno@geicam.org
Statistical Team	Mª José Escudero and María Isabel Casas CIBOMA Avenida de los Pirineos 7, oficina 1-14 28700 San Sebastián de los Reyes (Madrid) Spain Tel: +34916592870 Fax:+34916510406 E-mail: mjescudero@ciboma.org; micasas@ciboma.org
Biological sample management coordination	César García CIBOMA Avenida de los Pirineos 7, oficina 1-14 28703 San Sebastián de los Reyes (Madrid) Spain Tel: +34916592870 Fax:+34916510406 E-mail: cgarcia@ciboma.org
Central laboratory for analysis of physiological samples	CIBOMA main offices Avenida de los Pirineos 7, oficina 1-14 28703 San Sebastián de los Reyes (Madrid) Spain Tel: +34916592870
Experimental drug and control	**Control arm: Observation** **Experimental arm:** Capecitabine 1000 mg/m^2 twice a day for 14 days, followed by a rest period of 7 days, for 8 cycles.
Clinical trial phase	This is a Phase IV.III clinical trial. It is considered Phase IV because the study drug to be used has a marketing license in all the participating countries. However, the study product will be administered in an indication not approved in the marketing license, with the aim of evaluating its efficacy in increasing disease-free survival (Phase III study design).
Objectives	**Principal objective:** Compare disease-free survival after maintenance therapy with 8 cycles of capecitabine (X) compared to observation, in patients with operated, hormone receptor and HER2neu negative breast cancer who have received standard (neo-) and/or adjuvant chemotherapy. **Secondary objectives:** • Compare the 5-year Disease-Free Survival (DFS).

	• Compare Overall Survival (OS) between the two above-mentioned groups • Compare toxicity between the two above-mentioned groups. **Tertiary objectives:** Determine the effect of treatment with capecitabine on the development and duration of amenorrhea in premenopausal women. **(In selected centers):** Study the presence of thymidylate synthase (TS) and methylenetetrahydrofolate reductase (MTHFR) polymorphisms and confirm their relation with toxicity and efficacy of treatment with capecitabine.
Study design	Phase IV.III prospective, open, randomized treatment study. At the time of inclusion, after completing 6 cycles of standard (neo-) and/or adjuvant chemotherapy with anthracyclines and/or taxanes (**NOTE**: Patients without axillary node involvement may receive four cycles of adriamycin and cyclophosphamide (AC) as single chemotherapy treatment). The stratification will be carried out according to participating hospital, prior to chemotherapy (anthracyclines vs. anthracyclines and taxanes) and the number of ipsilateral axillary nodes involved (0, 1-3, ≥4), and the phenotype (basal vs. triple negative) and will be distributed randomly to receive: X x 8: capecitabine 1000 mg/m^2 p.o. administered twice a day (morning and evening) for 14 days, followed by a 7-day rest period, for 8 cycles. Observation Dose reduction and treatment delay and interruption have been planned in case of severe hematological or non-hematological toxicities. Indication for radiation therapy - Both groups: Patients will receive radiotherapy following the guidelines of each institution. Radiation therapy protocol/guidelines will be collected from each site. Not more than 4 weeks should elapse between the end of radiation therapy and patient registration of the patients in study CIBOMA/2004-01. Estrogen and progesterone receptor status: Estrogen and progesterone receptor status must be analyzed from a sample of the patients' primary tumor in the designated central laboratory. The results should be known before randomization. Any tumor which is not considered definitely negative, ie. on the limit, will be considered positive. HER2 expression status: An immunohistochemical analysis of the HER2 protein expression status must be made in the designated central laboratory. For tumors with a result of 2+, the number of copies of the c-erbB2 gene from the patients' primary tumor must be determined by FISH. Baseline genotype profile: An immunohistochemical analysis of cytokeratins CK5/6 and EGF receptor (EGFR) expression status must be performed in the designated central laboratory.
Disease or disorder under study	Patients with operated breast cancer with no metastatic involvement (AJCC, 2002). Patients will be able to participate whether they present axillary node involvement (node positive) or not (node negative). Node-negative patients must have a tumor greater than or equal to 1 cm diameter. At least six axillary nodes must be studied. For patients undergoing biopsy of the

	sentinel node, this node will count for the purposes of the number of nodes removed and with disease involvement. The patients' tumors must be estrogen and progesterone receptor negative and HER2 negative, according to the determination of the designated central laboratory.
Principal endpoint	Disease-free survival (DFS).
Inclusion criteria	1. Informed consent must be obtained and documented in writing before any protocol-specific procedures are performed, including the expected cooperation of patients in treatment and follow-up, in accordance with the ICH guidelines for Good Clinical Practice. The patient must sign informed consent for sending her tumor sample to the central laboratory. Subsequently, if the patient is eligible, a consent form must be signed for participation in the clinical trial. 2. Histologically-proven breast cancer (histological examination: invasive adenocarcinoma). 3. Patients with ispilateral axillary node involvement. If the sentinel node technique is used, the sentinel node will count as a resected involved node. Patients who can be classified in the following groups (AJCC 2002) will be admitted: - pN1a: Metastasis in 1 to 3 axillary nodes. - pN2a: Metastasis in 4 to 9 axillary nodes (at least one tumor deposit >0.2 cm). - pN3a: Metastasis in 10 or more axillary nodes (at least one tumor deposit >0.2 cm). Patients with a diagnosis of pN3a with infraclavicular node metastasis will **not** be eligible. **Nota:** Patients without axillary node involvement (N0), provided the primary tumor is greater than or equal to 1 cm in diameter. If the sentinel node technique is used, lymphadenectomy will not be necessary. 4. Out patient women aged≥ 18 years. 5. Karnofsky performance status ≥80 (ECOG 0,1).
Exclusion criteria	1. Definitive surgical treatment for operable breast cancer (T1-3, M0) must be mastectomy or breast-conserving surgery. The margins of the sample extracted in definitive surgery must be histologically free of invasive adenocarcinoma and ductal carcinoma *in situ* (DCIS). Lobular carcinoma *in situ* is not considered as a positive margin. 2. (Neo-) and/or adjuvant chemotherapy treatment with anthracyclines and/or taxanes (paclitaxel, docetaxel) of at least 6 cycles has not been received. **NOTE**: For patients without axillary node involvement that have not received at least four cycles of adriamycin and cyclophosphamide as single chemotherapy treatment. The allowed treatments are available in appendix 1. Any other treatment schedule should be approveb by the study medical coordinators. 3. In lymphadenectomy, resection of less than 6 nodes (For patients undergoing biopsy of the sentinel node, if it is positive, will count as a resected). 4. Previous treatment with anthracyclines or taxanes (paclitaxel, docetaxel) for any neoplasm other than the breast cancer being treated. 5. For patients that receive radiation therapy, no more than 8 weeks (2 months) should elapsed between day 1 of last (neo-) and/or adjuvant chemotherapy cycle and the clinical trial entry. For those patients in which the administration of adjuvant radiation therapy, more that 4 weeks (1 month) can be elapsed between the last session and the clinical trial entry.

	For patients that receive antineoplastic treatment before surgery, the interval between definitive surgical treatment and clinical trial entry is longer than 60 days. 6. Patients with unknown or positive estrogen, progeterone and HER2 receptor tumors. 7. Pregnant or nursing patients. Patients of child-bearing potential must have a negative result in a urine pregnancy test with the 14 days before treatment assignation. 8. Women in a fertile age that do not want to use a reliable and suitable contraceptive method. The postmenopausal women should have been with amenorrhea during at least 6 months to be considered as infertile (see section 6.5). 9. Diagnosis of invasive bilateral breast cancer. 10. Clinically relevant heart failure, such as congestive heart failure or symptomatic coronary artery disease, heart arrhythmia uncontrolled with treatment or history of myocardial infarction during the year before study inclusion, or uncontrolled hypertension. 11. History of significant neurological or psychiatric disease, including psychotic disorders, dementia or attacks which would impede the patient's understanding and giving informed consent, will interfere in compliance of the study drug regimen. 12. Uncontrolled active infection or other diseases or serious medical pathologies, such as active peptic ulcer, unstable diabetes mellitus. 13. Presence of anomalies in any laboratory parameter, as defined next: (in the previous 14 days to the treatment assigment): • Hemoglobin < 10 mg/dl; absolute neutrophil count < 1,5 x 10^9/l; platelet count < 100 x 10^9/l. • ASAT (SGOT) and ALAT (SGPT) > 2,5 ULN; alkaline phosphatase > 5 ULN; total bilirrubin > 2.0 ULN. Patients with ASAT and/or ALAT values >1.5 x ULN **associated** with an alkaline phosphatase level >2.5 x ULN will not be selected for the study. • Severe renal impairment , defined as creatinine clearance below 30 ml/min, according to the Cockroft and Gault formula (see appendix 9) or serum creatinine > 1,5 ULN. 14. Previous or present history of neoplastic disease other than breast cancer, with the exception of: • Non-melanoma skin cancer, cervical cancer in situ or other form of tumor curatively treated and without signs of disease for at least 10 years. • Ipsilateral ductal carcinoma *in situ* (DCIS) of the breast. • Lobular carcinoma *in situ* (LCIS) of the breast. 15. Known hypersensitivity to capecitabine, doxifluridine, fluorouracil or any of its excipients. 16. Patients with lack of upper gastrointestinal tract physical integrity or malabsorption syndrome, or with impossibility to take medication administered orally. 17. Previous treatment with capecitabine or with continuous infusion (> 24 h) with 5-FU, or with other oral fluoropyrimidines, such as eniluracil/5-FU, uracil/tegafur, S1 or emitefur. 18. Blood transfusions or administration of growth factors to help the hematological recovery in the 2 weeks prior to the beginning of the study treatment.

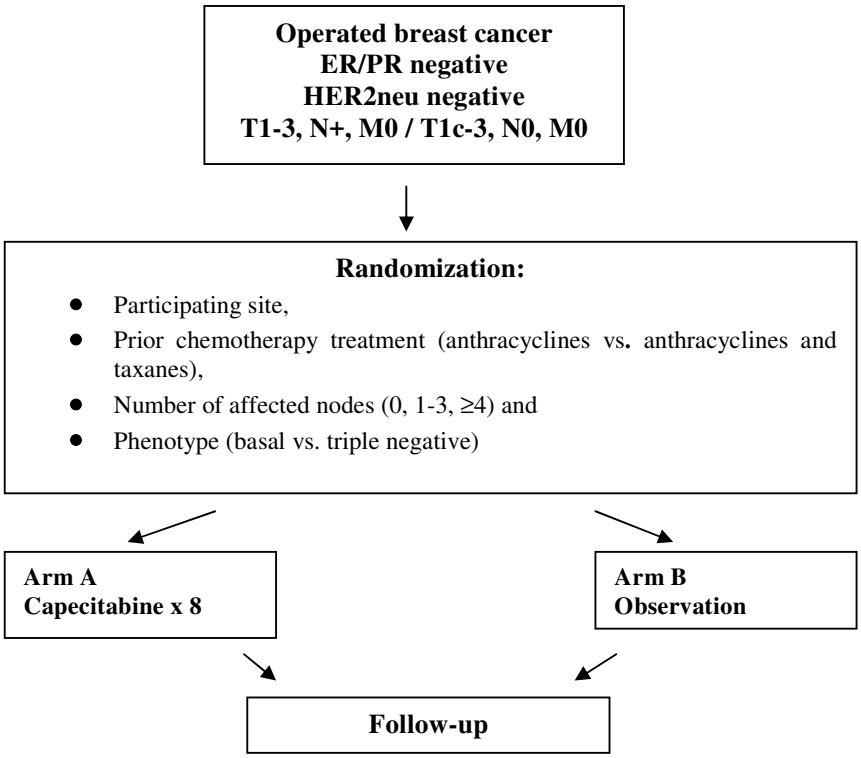
	19. Hormone treatment in the 10 days prior to the beginning of the study treatment. 20. Possibility of unexpected severe reaction in combination therapy with fluoropyrimidine, with or without proven dihydropyrimidine dehydrogenase (DPD) deficiency. 21. Allograft transplants of organs that require immunosuppressive therapy. 22. Patients treated with coumarin–derived anticoagulants. 23. Patients treated with sorivudine or chemically related analogs, such as brivudine. 24. Concomitant treatment with other investigational drugs. Participation in another clinical trial with any unmarketed investigational product during the 30 days prior to study inclusion. 25. Concomitant treatment with any other anticancer therapy. 26. Inaccessible patients for the treatment and monitoring. The patients registered on this trial should be treated and monitored in the participating center, that could be the center of the principal investigator or co-investigator. 27. Patients that cannot or do not want to comply with the protocol, considering the total duration of the study.
Study population and total number of patients	Assuming an exponential distribution, the aim is to detect an increase from 64.72% to 73.7% in 5-year disease-free survival (DFS), corresponding to a hazard ratio of 0.701 and a risk reduction of approximately 30%, with a power of 80% at a two-sided level of 0.05, considering 4 years of recruitment and 3 of follow-up. We would need to see a total of 255 events for DFS, 834 patients without considering drop-outs.. Considering a drop-out rate of 5% post-randomization, the final sample size will be 876 patients, 438 per treatment arm. The sample size calculation has been completed using the EAST statistical software, version 5.2. The randomization process will be centralized and patients will be stratified according to participating center, prior chemotherapy treatment (anthracyclines vs. anthracyclines and taxanes), and the number of involved nodes (0 vs 1-3 vs $\geq$4) and the phenotype (basal vs. triple negative).
Treatment duration	Planned treatment duration with X in the study treatment arm is 24 weeks. The same visit schedule will be followed for patients in the observation arm.
Timing and planned completion date	**Initiation of inclusion:** October 2006. **Completion of inclusion:** October 2010. **Clinical follow-up:** 5 years. **Final analysis:** Fourth quarter 2013.

STUDY DESIGN CIBOMA/2004-01:

This is a prospective, randomized clinical trial designed to evaluate the efficacy and safety of maintenance therapy with capecitabine in women with operated, hormone receptor and HER2neu negative breast cancer, who have previously received standard (neo-) and/or adjuvant chemotherapy. Node-negative patients are eligible if they have a tumor size greater than or equal to 1 cm.

Control treatment is observation.

Study treatment consists of the administration of 8 cycles of capecitabine at a dose of 1000 mg/m^2, twice a day, administered p.o. for 14 days, followed by a week's rest. The study will be carried out according to the following schedule:



Description of study population

Patients with operated breast cancer and no metastatic involvement (AJCC, 2002). Patients will be eligible whether they present axillary node involvement (node positive) or not (node negative), but node-negative patients must have a tumor greater than or qual to 1 cm diameter.

At least six axillary nodes must be studied. For patients undergoing biopsy of the sentinel node, this node will count for the purposes of the number of nodes removed and with disease involvement.

Only patients with estrogen and progesterone receptor negative and HER2 negative tumors will be eligible.

Estrogen and progesterone receptor status: An analysis of estrogen and progesterone receptors must be carried out using a sample of the patients' primary tumor which will be sent to the designated central laboratory. The best time for sending the tumor sample is at the beginning of standard chemotherapy treatment. Tumors which cannot definitively be considered negative, i.e. on the limits, will be considered positive. Only patients whose tumor samples are estrogen and progesterone negative will be eligible for the study.

The quantification system used will be a consensus between the UK NEQAS, SBCPG & EORTC from the year 2000:

Proportion of staining		**Intensity**	
0	No nuclear staining	0	No staining
1	≤ 1%	1	Slight staining
2	1-10%	2	Moderate staining
3	11-33%	3	Intense staining
4	34-66%		
5	67-100%		

The percentage of stained cells is evaluated and scored; the intensity of staining will be evaluated and another score will be applied; the final score will be the sum of both scores. Patients with tumors with no cell staining or slight staining (≤1%) will be eligible.

HER2 expression status: HER2 protein expression status will be analyzed by immunohistochemistry, from the sample of patients' primary tumor, which will be sent to the designated central laboratory. In the tumors with result 2+, the number of copies of the c-erbB2 gene will be determined by FISH, from the sample of patients' primary tumor.

Cytokeratin CK5/6 expression status: cytokeratin CK5/6 expression status will be analyzed by immunohistochemistry on the sample of patients' primary tumor sent to the designated central laboratory. The result will be correlated with hormone receptor, HER2 and HER1 (EGFR) receptor expression status, to establish concordance or divergence between the triple negative genotype and the baseline genotype.

HER1 (EGFR) expression status: HER1 protein expression status will be analyzed by immunohistochemistry on the sample of patients' primary tumor sent to the designated central laboratory. The result will be correlated with hormone receptor, HER2, cytokeratin CK 5/6 expression status, to establish concordance or divergence between the triple negative genotype and the baseline genotype.

2. INDEX

PROTOCOL SIGNATURE PAGE

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER
CIBOMA/2004-01
Chemotherapy vs. Observation

Approved by:
President of the Iberoamerican Coalition for Breast Oncology Research (CIBOMA)
Prof. Dr. Miguel Martín

_____ _____
Signature: **Date:**

Approved by:
Coordinator of Spanish Investigators
Dr. Ana Lluch

_____ _____
Signature: **Date:**

Approved by:
Coordinator of American Investigators
Dr. Laura Torrecillas

_____ _____
Signature: **Date:**

Approved by:
Coordinator of American Investigators
Dr. Carlos H. Barrios

_____ _____
Signature: **Date:**

DECLARATION OF PRINCIPAL INVESTIGATOR AND TEAM

With reference to the protocol:

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER
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I agree that this protocol contains all the necessary details for carrying out the study. I will carry out the study as explained in this document and in compliance with Good Clinical Practice Guidelines and with the Declaration of Helsinki (www.wma.net). I will endeavor to conclude recruitment of patients for the study by October 2010. I will provide copies of the protocol and all data on the drug regarding prior preclinical and clinical experience provided to me by CIBOMA to all doctors working under me who are participating in the clinical trial. I will discuss this material with the doctors to ensure that they are duly informed about the drug and the performance of the study. I agree to keep a record of all patient data (case report forms and patient informed consent), forms for receipt and return of drug, and other information collected during the study, in accordance with the legal requirements.

_____
Investigator (NAME IN BLOCK CAPITALS)

_____
Signature of Investigator Date

_____
Co-investigator (NAME IN BLOCK CAPITALS)

_____
Signature of Co-investigator Date

_____
Co-investigator (NAME IN BLOCK CAPITALS)

_____
Signature of Co-investigator Date

1. JUSTIFICATION

1.1 Background

Breast cancer is the most common type of cancer in women throughout the world. In 1999, more than 796,000 new cases were diagnosed and 314,000 deaths registered due to the disease (14.1%).
In the year 2000, 182.000 new cases (30.4% of all cancers in women in the U.S.) and 40,800 deaths (15.2% of all deaths due to cancer) were registered in the United States. It is calculated that approximately 30% of patients affected in the United States will die of the disease, and that 1 in 8 women will develop breast cancer at some stage in their lives.
In the European Community, 220,836 new cases were registered in 1997, which represents 51.67 cases of breast cancer per 100,000 inhabitants and 74,984 deaths (16.06 breast cancer deaths per 100,000 inhabitants). It is estimated that 1 in 10 European women will develop breast cancer at some stage in their lives.
In Spain, in 1997, 15,906 new cases of breast cancer were reported, which constitutes 69.98 cases of breast cancer per 100,000 inhabitants, with 5,766 deaths (22.67 per 100,000 inhabitants). The authorities calculate an annual mortality rate due to breast cancer of 29.3 per 100.000 women. It is estimated that 1 in 20 Spanish women will develop breast cancer before the age of 75.
Breast cancer is the principal cause of cancer death in Spanish women, with an adjusted mortality rate of 17.4%.
In Mexico, it is estimated that by 2010 the mortality rate will be 13 per 100,000 adult women and there will be about 4,500 breast cancer deaths a year.
In Columbia, breast cancer represents the third cause of death due to malignant tumors, after gastric cancer and cervical cancer. The weighted incidence in women aged between 30 and 50 is 80 per 100,000 women and 80% of the cases are diagnosed at a late stage, with a limited life expectancy.
In Cuba, one woman in 9 can expect to suffer this disease, with breast cancer constituting the principal cause of death due to cancer in women in this country.
In Peru, 7,837 deaths due to malignant processes among women were registered in the year 2000, with 736 breast cancer deaths.
A progressive increase in the incidence of breast cancer has been observed in Venezuela, which is only overtaken by cervical cancer. The estimated incidence rate is 28.08 per 100,000 women; the estimated cumulative rate is 3.10%, which indicates that 1 in 33 women in Venezuela will develop breast cancer during the course of her life. In 2000, 1,107 deaths due to this disease were registered and 2,704 cases were diagnosed. It is situated in the third place for death due to cancer, with 9.22 deaths per 100.000 women.
In Argentina, an increase in breast cancer mortality has been detected, from 20.9 per 100,000 women, recorded in 1988 to 26.7 per 100,000 women during the period 1997-2000.
In Costa Rica, the incidence recorded for breast cancer in 1998 was 9 per 100,000 women.
All these data come from official bodies. It seems clear that breast cancer has become a healthcare, social and economic problem of the first order in all countries, or will be soon.

1.2 Adjuvant chemotherapy in the treatment of breast cancer

Surgery is the primary therapeutic option for patients with breast cancer. Surgery and/or radiation therapy can control locoregional disease in the majority of patients. However, more than 60% of affected patients will finally die as a result of disease dissemination[1].

In recent years, there has been an increase in the administration of adjuvant hormone or cytostatic therapies. Studies ongoing at the moment show that adjuvant treatment can prolong time to disease recurrence and probably also survival in some patient groups[2,3].

Adjuvant chemotherapy is chemotherapy administered after primary surgery, with the aim of controlling clinically occult micrometastasis.

Although the optimal protocol has not yet been identified, various chemotherapy regimens have demonstrated their efficacy in the adjuvant treatment of breast cancer. They are grouped principally into regimens without anthracyclines, such as CMF (cyclophosphamide, methotrexate, 5-FU) and anthracycline-containing regimens, e.g. AC (doxorubicin, cyclophosphamide), CAF (cyclophosphamide, doxorubicin, 5-FU), FAC (5-FU, doxorubicin, cyclophosphamide), AVCF (doxorubicin, vincristine, cyclophosphamide, 5-FU) or FEC (5-FU, epirubicin, cyclophosphamide)[4-10].

Several randomized clinical trials have been performed comparing the CMF regimen or its variants, with cyclophosphamide-containing combination chemotherapies. The superiority of combination chemotherapy with anthracyclines appears to be real, but modest[6-10]. The results of the metaanalysis carried out by the Early Breast Cancer Trialists' Collaborative Group have confirmed that in general, both in patients with negative axillary lymph nodes and in those with positive axillary nodes and high-risk criteria, adjuvant chemotherapy significantly improves disease-free survival and overall survival[11].
Among the conclusions which can be drawn from the study results, it has been confirmed that it is better to administer 6 treatment cycles than 3.
Among new chemotherapeutic agents which emerged in the 90s, the taxanes are notable for their efficacy. Nevertheless, although the results tend to suggest that these agents could represent a significant advance in the management of breast cancer, their impact on the natural course of the disease remains to be defined.

Two different strategies have been adopted in the evaluation of the potential role of the taxanes in adjuvant treatment, as reflected in several large Phase III clinical trials:

a) The first strategy is associated with the concept of sequential chemotherapy, and is being investigated for both paclitaxel and docetaxel. Some cooperative groups which have performed clinical trials using this strategy are the CALGB (Cancer Leukemia Group B) with the ATC regimen (doxorubicin followed by paclitaxel followed by cyclophosphamide), the MD Anderson group with TFAC (paclitaxel followed by FAC), the CALGB and NSABP (National Surgical Adjuvant Breast and Bowel Project) with AC followed by paclitaxel or docetaxel, the Breast Adjuvant Study Team and the IBCSG (International Breast Cancer Study Group) with AT (docetaxel) followed by CMF, the French Cooperative Group with the FEC regimen (5-fluorouracil, epidoxorubicin, cyclophosphamide) followed by docetaxel, the BCIRG (Breast International Research Group) with AC followed by docetaxel $\pm$ trastuxumab (in HER2neu positive patients) and GEICAM with FEC followed by weekly paclitaxel[12].

b) The second strategy is based on the classic concept of combination chemotherapy, in which almost exclusively docetaxel-based combinations are studied. Comparative protocols with TAC (docetaxel, doxorubicin, cyclophosphamide) compared to FAC (BCIRG-001)[15], carried out by the BCIRG in axillary lymph node positive patients, or the protocol comparing the AT regimen (docetaxel) with AC, coordinated by the ECOG (Eastern Cancer Oncology Group) in patients with 1 to 3 positive nodes or with negative nodes and high-risk criteria. In addition, trials are being carried out to study whether triple combination is better than sequential chemotherapy, for example, the work coordinated by the BCIRG (BCIRG-005) in which TAC is compared to AC followed by T in node-positive, HER2-negative patients.

1.3 Adjuvant chemotherapy in operable node-positive, or node-negative, high-risk breast cancer

The most recent results from two of the combination regimens with taxanes have shown greater efficacy in the population of patients with operable breast cancer. These are the dense-dose regimen of bi-weekly AC followed by paclitaxel, based on the results of the CALGB-9741 study, and the TAC schedule (docetaxel, doxorubicin, cyclophosphamide), based on the results of the BCIRG001 study. Indirect comparison of the results of both studies suggests similar efficacy and tolerance, and both show an increase in overall survival, compared to standard regimens. In both cases, these are multicenter studies with large sample sizes, so the results can be considered as type I scientific evidence.

Study CALGB-9741[14]

Two thousand and five (2,005) node-positive patients were included with the aim of determining whether the dose density of the drugs would improve disease-free survival and overall survival. Patients were randomized to 4 arms, 2 sequential and 2 concurrent: Ax4 → T (paclitaxel) x4 → Cx4 (one arm had the cycles administered every 2 weeks and the other every 3 weeks), and ACx4 → Tx4 (one arm had the cycles administered every 2 weeks and the other every 3 weeks). The results after 36 months of follow-up in the CALGB-9741 study showed an increase in disease-free survival and in overall survival for the dose-dense regimen (A→C→P biweekly) of 85% vs 81% (p=0.01) and 92% vs. 90% (p=0.013) respectively, compared to the conventional treatment regimen (administration every 21 days).

Study BCIRG 001[15]

In the BCIRG 001 study, 6 cycles of TAC (docetaxel) were compared to 6 cycles of FAC in 1,491 node-positive patients. The results after 55 months of follow-up of study BCIRG 001 confirm the superiority of the TAC regimen in overall survival, compared to FAC (odds ratio 0.7 [0.53-0.91], p=0,0080). With regard to disease-free survival, a subgroup analysis (number of affected nodes, hormone receptor status) produced the following results:

N= 1,491	Odds ratio (Hazard Ratio)	P value
DFS		
All patients	0,72 (0,59-0,88)	0,0010
1-3 nodes (n=923)	0,61 (0,46-0,82)	0,0009
≥ 4 nodes (n=568)	0,82 (0,63-1,08)	0,1629
HR positive	0,73 (0,57-0,94)	0,0132
HR negative	0,66 (0,47-0,93)	0,0163

As can be observed, the patients who benefited most from TAC treatment are those with the least number of affected nodes and hormone receptor negative patients.

The study results described above have served as a basis for a new generation of randomized Phase III clinical trials which are currently ongoing, and are summarized below:

US Oncology 01-062 Study

This study will recruit 1,810 patients with operable, node-positive or node-negative breast cancer and high-risk criteria. Eligible patients are randomized to receive 4 cycles of AC followed by 4 cycles of docetaxel (ACx4→T4), compared to 4 cycles of AC followed by 4 cycles of docetaxel and capecitabine (ACx4→TXx4)

SWOG-50221 Study

This study will recruit 4,500 patients with operable, node-positive or node-negative breast cancer and high-risk criteria. Eligible patients are randomized to receive one of the following four treatment arms:

- 6 cycles of bi-weekly AC followed by 6 cycles of bi-weekly paclitaxel (ACX6→Px6)
- 15 cycles of weekly AC (with oral cyclophosphamide on days 1-7) followed by 6 cycles of bi-weekly paclitaxel (ACx15→Px6)
- 6 cycles of bi-weekly AC followed by 12 cycles of weekly paclitaxel (ACx6→Px12)
- 15 cycles of weekly AC with oral cyclophosphamide on days 1-7) followed by 12 cycles of weekly paclitaxel (ACx15→Px12)

NSABP B-38 Study

This study will recruit 4,500 patients with operable, node-positive breast cancer. Eligible patients are randomized to receive one of the following three treatment arms:

- 6 cycles of TAC (docetaxel, doxorubicin, cyclophosphamide) every 21 days (TACx6)
- 4 cycles of bi-weekly AC followed by 4 cycles of bi-weekly paclitaxel (ACx4→Px4)
- 4 cycles of bi-weekly AC followed by 4 cycles of bi-weekly paclitaxel/gemcitabin (ACx4→PGx4).

NCIC-CTG MA.21 Study

This study will recruit 1,500 patients with operable, node-positive or node-negative and high-risk breast cancer. Eligible patients are randomized to receive one of the following three treatment arms:

- 6 cycles of epirubicin and fluorouracil (days 1-8) with oral cyclophosphamide (days 1-14) every 28 days (FECx6)
- 6 cycles of bi-weekly EC followed by 4 cycles of paclitaxel every 3 weeks (ECx6→Px4)
- 4 cycles of AC every 3 weeks followed by 4 cycles of paclitaxel every 3 weeks (ACx4→Px4)

CALGB 49907

This is a phase III trial which will include patients ≥65 years of age with operable, node-positive or node-negative, high-risk breast cancer. The aim is to offer a viable alternative to this large group of older patients who frequently receive suboptimal treatment for fear of increasing toxicity[42]. Patients will be randomized to one of the following arms:

- Oral CMF x 6 cycles
- AC x 4 cycles
- Capecitabine x 6 cycles

Study GEICAM/2003-10

This study will recruit 1,302 patients with operable, node-positive breast cancer. Eligible patients are randomized to receive one of the following two treatment arms:

- 4 cycles of epirubicin and cyclophosphamide every 21 days followed by 4 cycles of docetaxel every 21 days (ECx4→Tx4)
- 4 cycles of epirubicin and docetaxel every 21 days followed by 4 cycles of capecitabine on days 1-14 of every 21-day cycle (ETx4→Xx4)

All these studies have a series of criteria in common:

- The combination regimens of anthracyclines and cyclophosphamide are administered either in a dose-dense or in a metronomic regimen.

- A minimum of 4 cycles of chemotherapy are administered (doxorubicin + docetaxel) and the treatment periods tend to be extended, with an increase in the number of cycles administered.
- New products which have shown efficacy in the treatment of advanced or metastatic breast cancer (capecitabine, gemcitabin) are added to the drugs already well-known in adjuvant treatment (anthracyclines and taxanes).

1.4 Neoadjuvant chemotherapy in breast cancer[75-103]

Adjuvant chemotherapy is the only systemic treatment that has been administered to patients with triple-negative tumors. The absence of hormone receptors and HER2 expression means that the alternative hormone therapy and treatment with trastuzumab is not applicable.

Development of neoadjuvant treatment was based on treatment of women with locally advanced tumors, which could not undergo surgery, and they were treated with chemotherapy regimens with promising results. Some patients obtained good responses, which allowed a surgical procedure. Subsequently, a large number of cases have reported the use of neoadjuvant chemotherapy in patients with tumors with possibility of surgery. Initially, the objective was to obtain a response which would allow a surgical procedure keeping the breast. The results found in many cases allowed the use of this strategy as perfectly acceptable with this objective.

A second aspect of neoadjuvant treatment is that it allows in vivo evaluation of the tumor response, which has been related to the patient's prognosis and survival. A variable rate of 10-30% of complete pathological responses has been reported with a very clear association with improved survival compared to the survival of patients who do not respond, or who partially respond to treatment.

Thus, neoadjuvant treatment is currently established as an alternative, which should be discussed with the patient when her specific objectives apply to the specific situation. If the patient wishes to keep the breast and the tumor size does not allow it, chemotherapy treatment before surgery has the possibility of helping to reduce the tumor size and allow conservative surgery. One of the neoadjuvant approach limitations is that it has to take place before the surgical procedure, and we do not have pathological information regarding the number of axillary nodes involved. Demonstrating that results are equivalent when we assess survival parameters makes this limitation not to have a high impact on clinical management of patients.

One of the questions raised and analyzed in different clinical studies is associated with the comparison of results between treatment administration before or after surgery. It is very important to highlight that in none of the clinical trials that have been conducted up to now, neoadjuvant treatment obtained results inferior to adjuvant treatment. In a meta-analysis published by Mauri et al, it was clearly proven that the comparison of both treatments does not show differences in relation to disease-free survival and overall survival. In this sense, it is critical to consider that there is no biological reason that has been proven up to now, which justifies that the results of the same regimen, before or after surgery, are different.

1.5 Capecitabine

Capecitabine is an oral fluoropyrimidine carbamate which is tumor-activated via a cascade of 3 enzymes which supply high and prolonged levels of the active fraction, 5-fluorouracil (5-FU), within tumor cells. Fluorouracil is one of the most widely used cytotoxic agents, but its efficacy is limited by its lack of selectivity. Capecitabine was developed in the laboratory as a tumor-selective agent which would alter favorably the risk-benefit ratio, particularly in the context of advanced, resistant breast cancer[31].

1.6 Pharmacology of capecitabine

After oral administration, capecitabine is absorbed unchanged in the gastrointestinal tract and metabolized in the liver by 60kDa *carboxylesterase*, a 5'-DFCR. It is then converted to 5'-DFUR by *cytidine deaminase* which is found in the liver and in tumor tissues. Metabolism of 5'-DFUR to 5-FU is subsequently produced in the tumor itself, due to the action of *thymidine phosphorylase* (dTHdPasa). Consequently, the exposure of healthy tissue to systemic 5-FU is minimized.[16]

It was recently determined that the enzyme responsible for the final catalytic conversion of capecitabine to 5-FU (dThdPasa) has an identical structure and function to that of the tumor-associated angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF)[17-19]. PD-ECGF is a 55 kDa polypeptide which exists *in vivo* as a homodimer. It was originally isolated from the platelets as a endothelial mitogen and angiogenic factor[20,21]. PD-ECGF has been shown to be an important angiogenic factor in breast cancer. Its expression is correlated with the intensity of angiogenesis, an increase in tumor size and in the invasive capacity of the tumor, and with a worse outcome[21,22]. Tumor areas with poorer perfusion, defined by hypoxia and acidosis, have a greatly increased expression of PD-ECGF[20]. The fact that within the tumor, and in particular in more aggressive tumors, there are elevated levels of FD-ECGF/dThdPasa, responsible for the final activation of capecitabine, supports the antitumor efficacy of this drug.

1.7 Preclinical antitumor efficacy of capecitabine

Capecitabine demonstrated cytotoxicity only after conversion to 5'-DFUR and 5-FU. In 12 xenograft models of human cancers tested in mice, capecitabine was always more active than 5-FU. In addition, it showed activity in 5-FU-resistant xenografts. The therapeutic indexes were much more favorable than those observed with 5-FU. Antitumor efficacy in mice was correlated with tumor levels of 5-FU and blood levels of 5'-DFUR. In addition, also in mice, capecitabine showed anticachetic activity, by normalizing body weight, loss of adipose tissue and episodes of hypoglycemia. In this same model, capecitabine showed antimetastatic action at doses 40 to 50 times lower than those required for activity against the primary disease[16,23].

Preclinical studies have revealed that the antitumor activity of capecitabine depends on the total dose administered, so that the regimen (uninterrupted daily administration; days 1-5 of each week; days 1-14 every 3 weeks) does not influence its antitumor activity[24].

1.8 Phase I clinical studies with capecitabine: regimens tested, maximum tolerated dose and recommended dose

The maximum tolerated dose (MTD) for capecitabine in continuous or intermittent monotherapy[25-27] or in combination with leucovorin[28] was defined in four Phase I studies (SO14693, SO14794, JO14865, SO14798). The recommended doses determined by these studies were later used with satisfactory results in phase II and III studies. In general the three regimens used (continuous, intermittent or combined with leucovorin) showed significant antitumor efficacy, including complete responses. The three regimens tested were tolerable with very few grade 3/4 events, all manageable with dose modification and/or symptomatic intervention. The overall evaluation carried out by the investigators and the sponsor based on these phase I studies and on a randomized phase II study in colorectal cancer[29] was that the intermittent regimen was the most promising, being associated with a reasonable toxicity profile and longer time to disease progression, while at the same time allowing the administration of a higher intensity of dosing to the patient (approximately 25% more than the continuous regimen). The additional advantage of the "off drug" periods meant this regimen was also considered by the patients as more satisfactory.

From the European phase I study with an intermittent regimen (2 weeks treatment and one week rest), a MTD was defined to be 1500 mg/m^2 twice a day, while the dose recommended for the phase II studies was established at 1250 mg/m^2 twice a day[26]. In this same study, which included heavily pre-treated patients, seven with breast cancer and 17 with colorectal cancer,

four objective responses were described (1 CR and 3 PR). The tumors which responded were breast, esophagus, colon and rectal cancers.

A Phase I treatment study of the combination of capecitabine with paclitaxel[30] defined a recommended dose for phase II studies of oral capecitabine 1331 mg/m^2/day, divided in 2 daily doses administered continuously, and 175 mg/m^2 paclitaxel in a 3 hour IV infusion, every three weeks. No clinically relevant pharmacokinetic interactions were observed between both drugs. No responses were observed (15 of 17 patients were clearly resistant to 5-FU and 13 of 17 patients had cancers considered intrinsically resistant to taxanes).

Phase I study SO15304 investigated the combination of capecitabine administered on days 1-14 together with docetaxel administered on day 1 of each 3-week cycle[31]. The progressive dose increase was applied in two phases. Initially the dose of docetaxel was increased (75, 85 and 100 mg/m^2) in combination with a fixed dose of capecitabine (1650 mg/m^2/day). Then the dose of capecitabine was increased (2000 and 2500 mg/m^2/day) in combination with a fixed dose of docetaxel, defined as tolerable in the first phase. Thirty-three (33) patients were included (15 men, 18 women) distributed over seven dosing levels: docetaxel 75 (4 patients), 85 (6 patients) and 100 (6 patients) mg/m^2 combined with capecitabine 1650 mg/m^2/day; docetaxel 100 (5 patients) mg/m^2 combined with capecitabine 2000 mg/m^2/day; and then capecitabine 2000 (6 patients) and 2500 (6 patients) mg/m^2/day combined with docetaxel 75 mg/m^2. Uncomplicated grade 4 neutropenia (afebrile and duration less than 7 days) was observed at all dosing levels. One case of grade 3-4 neutropenia associated with grade 2 fever was observed in the final treatment cycles of each of the dosing levels 85/1640, 100/1650 and 100/2000 mg/m^2 docetaxel/capecitabine. One patient with dosing levels of 100/2000 and 85/1650 mg/m^2 docetaxel/capecitabine experienced mucositis NCIC/CTC grade 3. The five patients treated at the dose level of 100/2000 mg/m^2 docetaxel/capecitabine reported grade 2 chronic asthenia and fulfilled the definition of MTD. Other common toxicities (grade 2) were alopecia, diarrhea, mucositis, hand-foot syndrome/skin toxicity, nail dystrophy, nausea and vomiting. Dose reductions were required in 7 patients, 5 due to hand-foot syndrome or nail dystrophy, one due to stomatitis and one due to thrombocytopenia. Partial responses were observed in three out of four patients with breast cancer and minor responses in adenocystic submandibular carcinoma, non-small cell lung cancer, colorectal carcinoma and an adenocarcinoma of unknown primary origin. Minimal toxicity other than the expected neutropenia was observed at a dose level of 75/2500 mg/m^2 docetaxel/capecitabine, with only two patients experiencing short-term grade 2 asthenia (which occurred during a hold in dexamethasone co-medication). As such, this dose level was selected for subsequent studies.

1.9 Phase I clinical studies with capecitabine: Pharmacokinetics of capecitabine in humans

The pharmacokinetic parameters for the product were obtained from phase I studies. The communication from Twelves[32] summarizes the majority of the data determined to date. These data are based on 32 patients who received doses of 502 to 2510 mg/m^2/day separated in two doses. Capecitabine absorption was rapid and practically complete. Peak plasma concentrations of the drug and of the two principal metabolites (5'-DFCR and 5'-DFUR) were reached rapidly (0.5 to 1.5 hours) after administration. Plasma protein binding of capecitabine and its metabolites was less than 60%. Concentrations then fell exponentially with a half-life of 0.5 to 1 hour. More than 70% of the administered dose of capecitabine was recovered in the urine. After administration of 829 mg/m^2, the greatest AUC was obtained for 5'-DFUR (11.8 µg.mL^{-1}.h, CV=44%, n=15). Logistic regression techniques were used to evaluate the relation between toxicity and systemic exposure. The two patients with the greatest exposure to 5'-DFUR had grade 3 toxicity. Similar pharmacokinetic parameters were obtained on days 1 and 15$\pm$1. The pharmacokinetic characteristics of capecitabine and its metabolites were dose-proportional at doses up to 1657 mg/m^2/day.

A correlation between the presence of food with a modification of some pharmacokinetic parameters (reduction in maximum plasma concentration and of AUC and increase in time to

maximum plasma concentration) compared to the administration of capecitabine in fasting conditions was observed in one study[33]. Until then, the other clinical studies were carried out in the presence of food. For this reason, and in the absence of safety/efficacy data for capecitabine administered in fasting conditions, the current recommendation is to continue administering the drug 30 minutes after ingestion of food (breakfast or lunch).

A pharmacokinetic study was carried out in 19 patients with colorectal cancer undergoing surgery with the aim of studying the differential concentrations of 5-FU (in tumor, healthy tissue and plasma) after administration of capecitabine. After oral administration of capecitabine (1255 mg/m^2 twice a day for 5 to 7 days before surgery), concentrations of 5-FU were significantly greater (mean geometric ratio 2.5; CI: 1.5 to 4.1) in the primary tumor than in normal adjacent tissue[34.] This finding is consistent with other studies. Thus, human tumors, in particular breast, gastric, colorectal, cervical and ovarian tumors, have shown much higher levels of dThdPasa (responsible for the conversion of 5'-DFUR to 5-FU) than corresponding normal tissue[16].

1.10 Capecitabine in monotherapy. Phase II breast cancer studies.

A Phase II study was carried out in 162 patients with metastatic and/or advanced breast cancer who had failed treatment with paclitaxel[35]. 90% of these patients had been treated previously with anthracycline-based regimens. In addition, 81% of the patients had received treatment regimens which included 5-FU. The average number of previous treatments was 2.5. One hundred and thirty-five (135) patients had bidimensionally measurable disease. Twenty-seven (27) had evaluable disease. The treatment schedule was capecitabine 2510 mg/m^2/day for 14 days, followed by a 7-day rest period. An objective response rate of 20% was observed (CI 95%: 14-28%) which was shown to be robust, according to the consistency of the results obtained over the different centers and patient subpopulations. Median duration of response in responders was 241 days. At the end of the observation period, disease progression had still not been observed in 11 of these patients. Median time to progression was 93 days. Median survival observed in all cases was 384 days, greater or equal to that obtained in currently available chemotherapies used as second or third line treatment. Treatment was relatively well tolerated, with very few drop-outs due to toxicity (8%). The most common adverse effects were, in order of frequency, hand-foot syndrome, diarrhea, nausea and vomiting and fatigue (see table below). Grade 4 diarrhea was detected in 4 patients. There were no toxic deaths. Rare (<5%) cases of grade 3-4 neutropenia were observed. Most significant among the laboratory findings was hyperbilirubinemia, which reached grade 3-4 in 17 patients (10.49%) although in 9 of these cases, it coincided with progressive liver disease. In the other 8 cases, hyperbilirubinemia was more moderate (grade 3, i.e. rise of total bilirubin of 1.5 to 3 x UNL) and transient, and did not require treatment withdrawal. In general, toxicity was predictable and manageable. Essentially, on the basis of these results, the Food and Drug Administration (FDA) approved capecitabine for treatment of metastatic disease after progression on anthracyclines and taxanes in September 1998.

Table: Summary of adverse effects related with capecitabine treatment (Blum, JCO 1999)

	Total (%)	*Severe(%)*	*Life-threatening (%)*
Hand-foot syndrome	56.2	9.9	_
Diarrhea	54.3	11.1	3.1
Nausea	51.9	4.3	_
Vomiting	37.0	3.7	_
Fatigue	36.4	7.4	_
Constipation	14.4	1.2	_
Dermatitis	15.4	1.2	_
Stomach pain	14.8	3.1	_

Loss of appetite	11.1	0.6	_
Fever	11.1	0.6	_
Erythematous rash	10.5	_	_
Paresthesia	9.9	_	_
Stomatitis	9.3	2.5	_
Inflammation of mucous membranes	9.3	4.3	_
Dehydration	6.8	3.1	_
Coagulation changes *	0.6	_	0.6

*prolonged prothrombin time in one patient treated with warfarin. Labeled by investigator as possibly related.

Another phase II study also carried out in heavily pretreated patients[36] (100% had received paclitaxel and/or docetaxel, 92% had received anthracyclines) using a very similar capecitabine regimen (1250 mg/m^2 twice a day, days 1-14, every 3 weeks). One hundred and five (105) patients were evaluable for toxicity, while 100 were evaluable for response. A response rate of 18% was observed (16% PR, 2% CR). Toxicity was generally moderate, grade 1-2, and consisted of hand-foot syndrome (42% of patients), nausea and vomiting (40%), diarrhea (26%), stomatitis (16%) and lethargy (15%). Grade 3 hand-foot syndrome was reported in 13% of patients. In addition, grade 3 emesis and diarrhea was observed in 5% and 3% of cases, respectively.

Jakob et al[37] treated 13 patients with the same capecitabine regimen. All patients had received anthracyclines, taxanes and high dose chemotherapy. A response rate of 54% (CI 95%:26-81%) was obtained. The median time to progression was 128 days. The toxicity spectrum was similar to that described in the previous studies. Five patients (37.5%) developed grade 3 hand-foot syndrome.

In another study carried out in patients previously treated with anthracyclines, paclitaxel at the usual doses (175 mg/m^2 every three weeks) versus intermittent capecitabine (2510 mg/m^2/day in two doses, days 1-14, every three weeks) was evaluated[38]. The study closed prematurely due to lack of recruitment (refusal for randomization). Finally only 22 patients received capecitabine and 20 paclitaxel. Eight responses were observed (36%, CI 17-59%), including 3 complete responses, in the group treated with capecitabineFour partial responses were observed in the paclitaxel group (21%, CI 95% 6-46%). The median time to progression was 92 and 95 days for capecitabine and paclitaxel, respectively. The proportion of patients with grade 3-4 toxicity was 22% in the capecitabine group and 58% in the paclitaxel group. The study, although notably limited by its small sample size, supported similar efficacy between both drugs, with a better tolerance profile for capecitabine.

Similarly, in a Japanese phase II study carried out in 50 patients previously treated heterogeneously (previous exposure to anthracyclines 36%, to alkylating agents 54%, to 5-FU 64%, and to hormones 74%), a response rate of 28.3% was described for the total group and 23.5% for the group pretreated with anthracyclines[39].

A randomized treatment study carried out in 95 treatment-naïve patients[40] compared capecitabine administered in an intermittent regimen to iv CMF administered on day 1 every three weeks. The response rate for capecitabine and CMF was 25% (CI 95%, 14-37%) and 16% (CI 95%, 5-33%) respectively. The median time to progression was greater with capecitabine (132 vs 94 days). A higher proportion of grade 3 toxicity was observed in the capecitabine group (132 vs 94 days). A greater proportion of grade 3 toxicity was observed in the capecitabine group (44 vs 20%). These differences were due essentially to a higher frequency of hand-foot syndrome (16 vs 0%) and diarrhea (8 vs 3%) in the group which received the oral regimen. No statistical analysis was made for any of the efficacy or toxicity parameters. The authors concluded that capecitabine showed at least similar efficacy to CMF when administered as first-line treatment.

Subsequent studies have confirmed the efficacy and tolerance of capecitabine treatment in metastatic breast cancer[43]. Capecitabine in combination with docetaxel has also been shown to be more effective in the treatment of metastatic breast cancer than docetaxel in monotherapy, in terms of survival and response rates[44].
Given the role of capecitabine in metastatic breast cancer as a highly effective agent with no cross resistances, and the advantage of oral administration, the study of its administration in the adjuvant area seems justified.

1.11 Capecitabine in the neoadjuvant treatment of breast cancer

Primary treatment of stage II/III breast cancer with a combination of docetaxel and capecitabine provides higher pathological and clinical response rates than other regimens, such as doxorubicin/cyclophosphamide (AC). In the study carried out by Hong Gi Lee et al[45], patients were stratified by disease stage (II vs III), estrogen receptor status and age. Patients were randomized to receive 4 cycles of AC (doxorubicin/cyclophosphamide 60/600 mg/m^2) every 21 days, or 4 cycles of TX (docetaxel 75 mg/m^2, day 1 of each 21-day cycle; oral capecitabine 1000 mg/m^2 twice a day, day 1-14 of each 21-day cycle). After the fourth treatment cycle, breast-conserving surgery or mastectomy was performed on the patients and there was a treatment cross-over. Patients could subsequently receive radiation therapy and tamoxifen, where indicated. An interim analysis was performed which included data from 53 patients in the TX arm and 47 in the AC arm. Patients in the TX arm presented a clinical response rate of 91% compare to 74% in the AC arm. Nine percent of patients in this arm had disease progression on treatment, which did not occur in the patients in the TX arm.
The following table summarizes the characteristics of patients who achieved complete pathological response in the breast and lymph nodes for each treatment group:

Characteristics	AC (n=47)	TX (n= 53)
Tumor		
Stage II/III	3/11	23/23
ER+/ER-	0/14	17/30
PgR+/PgR-	0/12	19/25
HER2 (3+/0-2+)	7/6	36/18
Total (% patients)	6%	23%
Lymph nodes		
Stage II/III	31/22	36/32
ER+/ER-	12/48	33/35
PgR+/PgR-	10/42	33/34
HER2 (3+/0-2+)	29/27	64/23
Total (% patients)	28%	34%
ER: estrogen receptor; PgR: progesterone receptor		

As can be seen in the table, the complete pathological response rate was higher in the TX treatment arm, both in breast (23% vs. 6%) and axillary nodes (34% vs. 28%). In addition, the TX combination seems to be equally effective in HER2 positive and negative patients.
With regard to the safety profile, patients who received AC experienced more episodes of nausea and vomiting, while patients treated with TX experienced more myalgia and skin and nail events.

1.12 Polymorphisms related with efficacy and effectiveness of capecitabine

5-Fluorouracil (5-FU) inhibits thymidilate synthase (TS) which catalyzes the conversion from uridilate to thymidilase. TS is the only *de novo* source of thymidilate, which is a precursor of the thymidilate triphosphate necessary for DNA synthesis. Attempts to administer 5-FU orally have failed due to its low and unpredictable bioavailability caused by its being metabolized by

dihydropyrimidine dehydrogenase (DPD) in the liver and intestinal walls[46-48]. This has led to the development of active oral analogs of 5-FU, such as capecitabine[49].

Capecitabine is a member of the drug family of the fluoropyrimidines. It is metabolized to 5'-deoxy-5-fluorocytidine by hepatic carboxylesterase. Histidine deaminase converts the 5'-deoxy-5-fluorocytidine to 5'-deoxy-5-fluorouridine. The tumor selectivity of capecitabine is due to the conversion of 5'-deoxy-5-fluorouridine to 5-FU by thymidine phosphorylase, an enzyme which is usually present in high levels in human tumors[50].

Takeishi et al[51] showed that the TS gene has a satellite in the 5' region which is not transcribed, which consists of a tandem of 3 repetitions (3R), each one 28 base pairs long. Subsequent research has shown that the promotor of this gene is polymorphic, with individuals which have only 2 repetitions (2R) and others which have up to 9 repetitions (9R)[52-54]. The number of repetitions in tandem affects the transcription of the TS gene[55]. Patients who are homozygous for three repetitions (3R/3R) have higher levels of TS protein than heterozygous 2R/3R patients. Using *in vitro* expression of genes with 2R and 3R, higher levels of the TS protein were obtained due to the better transcriptional efficacy of the 3R messenger DNA, compared to an increase in production of messenger DNA.

Various studies have suggested that 3R/3R TS genotypes are associated with a poorer response to 5-FU chemotherapy in colorectal cancer. It is suspected that the reason for this is that 3R/3R genotypes are associated with higher levels of TS protein, which confers resistance to 5-FU. In a retrospective analysis, in which patients with colorectal cancer were treated with capecitabine, response rates were 14% in the subjects with 3R/3R genotype, compared to a response rate of 80% in subjects with the 2R/2R genotype[56].

Recent studies have shown that TS tandem repeat polymorphisms contain potential binding sites called E-box (CACTTG) which bind transcription factors on the binding site for stimulatory factor 1 (USF-1) and stimulatory factor 2 (USF-2)[57]. In genotypes 3R/3R, there are two E-box binding sites in the second and third repeats, while in the 2R genotype, there is only one E-box binding site. The final repeat in both genotypes 2R and 3R in nucleotide 12 is imperfect, so while the first repeat is a G, it has been replaced here by a C. A polymorphism was discovered in position 12 G→C in the second repeat of the genotype 3R which inhibits the binding of transcription factors USF-1 and USF-2. The effects of this polymorphism on the efficacy or toxicity of drugs which inhibit TS activity must be determined in retrospective or prospective studies.

The most frequent toxicities associated with the administration of capecitabine are hand-foot syndrome, diarrhea, nausea, vomiting and mouth irritation. In general, capecitabine is well tolerated; the percentages of patients who present severe toxicities after the first cycle is 22% for hand-foot syndrome, 16% for diarrhea and 12% for mouth irritation. For patients with severe toxicity, the dose can be reduced in subsequent cycles, without a reduction in efficacy. Unfortunately there is no way of predicting which patients will experience greater toxicity. As TS genotypes are identical in primary colorectal tumors and in normal tissues, it could be expected that individuals with 3R/3R genotype would have increased expression of TS in normal tissues, which would protect them from capecitabine toxicity[58]. A small retrospective study of patients with colorectal cancer treated with different 5-FU-based regimens showed that overall grade 3 toxicity was 36% lower in individuals with 3R/3R polymorphisms compared to patients with 2R/2R[59]. The effects of polymorphisms on the risk of capecitabine toxicity in early-stage breast cancer are still unknown.

Methylenetetrahydrofolate reductase: MTHFR may also play an important role in the toxicity and efficacy of capecitabine. A single nucleotide polymorphism (SNP) in position 677 of MTHFR, due to a conversion from C to T, produces a thermolabile enzyme which is rapidly degraded[60]. The allele frequency of polymorphisms in MTHFR varies with geographical location and race. It is estimated that homozygosity for TT is present in approximately 10% of North Americans[61]. Individuals homozygous for TT have increased levels of methylenetetrahydrofolate, a compound with stabilizes the binding of 5-FU to TS[62], resulting in

the formation of the ternary complex of 5-FU, TS and methylenetetrahydrofolate. Our hypothesis is that the increased stabilization of this ternary complex can cause an increase in both capecitabine toxicity and efficacy.

A retrospective study carried out by Cohen et al[3] supports the hypothesis that MTHFR polymorphisms can influence toxicity and response to fluoropyrimidine-based chemotherapeutic treatment. In this study, 43 patients were treated with one of the following regimens: i) capecitabine twice a day for 2 weeks with one week rest, ii) tegafur plus uracil three times a day for 28 days with 7 days rest, iii) the Mayo Clinic 5-FU/LV regimen. Response rates and frequency of toxicities were combined for the three studies: wild-type homozygous MTHFR genotype (C/C) had a response rate of 47% compared to a response rate of 67% in MTHFR heterozygous individuals, and a 100% response rate in MTHFR T/T homozygous individuals. These differences were not statistically significant due to the small sample size. When the patients are analyzed according to the frequency of mutant alleles, patients with a T allele had a response rate of 77% compared to a 55% response rate in patients with a C allele. This comparison reached statistical significance.

MTHFR and folate interaction: MTHFR polymorphisms have been studied as possible risk factors in diseases such as neural tube defects in children[64], colon cancer[65], and ischemic heart disease[66]. The epidemiological studies of MTHFR as a risk factor in diseases measured folate levels in study subjects to determine whether they influenced the role of the MTHFR polymorphisms as risk factors. The doctors' health study[65] showed that the MTHFR polymorphism C677T conferred protection against the development of colon cancer only if patients had adequate folate levels. Christiansen et al[67] showed that women with MTHFR genotype TT had a higher risk of having a child with a neural tube defect, and that the risk increased significantly if the mother had a folate deficiency. In contrast, a study on the effects of the C677T polymorphism in methotrexate toxicity did not show any difference in patients on folate supplements or not. This study did not measure folate levels and used folate supplements as a substitute for folate level status.

Dihydropyrimidine dehydrogenase: Dihydropyrimidine dehydrogenase (DPD) deficiency has been identified as the cause of life-threatening reactions which appear rarely due to the administration of fluoropyrimidines[68,69]. Individuals with a complete deficiency of this enzyme have significantly raised levels of uracil and thymine in blood and urine. Etienne et al[70] studied the possibility of using DPD activity as a marker for predicting the risk of fluoropyrimidine toxicity, in a prospective study of 185 patients treated with 5-FU-containing regimens. They found a normal distribution of DPD activity among the patients, and no correlation between activity and 5-FU activity.

In conclusion, we are setting out from the hypothesis that TS and MTHFR polymorphisms can predict which patients will experiment toxicities and require dose reductions of capecitabine. In addition, TS and MTHFR polymorphisms could predict which patients would not benefit from the standard capecitabine dose. We speculate that the following genotypes will be found in the study: i) TS genotype 2R/2R (severe toxicity, benefit from adjuvant therapy, need for dose reduction; ii) TS genotype 2R/3R (intermediate toxicity, intermediate benefit from adjuvant therapy); iii) TS genotype 3R/3R (slight or no toxicity, no benefit from adjuvant therapy).

1.13 Basal-like breast cancer

The work of the various investigator groups has consolidated the development of a tumor classification for breast cancer based on three types of genetic profile: luminal tumors (hormone receptor positive), HER2 tumors (HER2neu positive) and basal-like tumors (hormone receptor

and HER2neu negative). The latter, also known as "triple negative" are the object of the present study protocol.

Basal-like tumors receive this name because their genetic expression profile is similar to that of a normal basal epithelial cell. These similarities include the absence of expression of the estrogen receptor and other genes related with this and the HER2 receptor. They also share with the basal epithelial cells overexpression of cytokeratins 5/6 and 17, EGFR and genes associated with proliferation. p53 mutations in thyrosine are also basal cell characteristics.

Numerous independent studies indicate that basal-like tumors have a poor outcome. Indeed, treatment is complicated, since patients with triple-negative tumors are not candidates for endocrine treatment or trastuzumab. However, this type of tumor is very sensitive to chemotherapy. Investigators at the MD Anderson Cancer Center examined the chemosensitivity of this type of tumor[71]. The genetic profile was obtained by fine needle biopsy in 83 breast tumors, before neoadjuvant treatment with paclitaxel and FAC. Complete pathological response was more frequent in basal-like tumors, compared to luminal-type tumors. This result was confirmed by a study from the University of North Carolina, in which the type of cancer was classified in 105 patients treated with AC and taxanes. Twenty-seven percent (27%) of the tumors were basal-like, 21% were HER2 positive and 52% were luminal. The best clinical responses to treatment with chemotherapy were obtained in patients with basal-like tumors (86%, compared to 68% in the HER2 positive patients, and 60% in the luminal tumors)[72]. In parallel, the complete pathological response rate was greater in patients with basal-like tumors (30%, compared to 27% in HER2 positive and 13% in luminal tumors).
Despite the greater sensitivity to chemotherapy, basal-like tumors have a poor outcome. With a median follow-up of 2.5 years in the University of North Carolina study, patients with basal-like tumors presented a clear tendency towards early recurrence. The reason appears to be the lack of a satisfactory therapeutic arsenal for the treatment of hormone receptor negative, HER2 negative patients, so that, if there is residual disease, outcome is poor.

The North American cooperative group, Cancer and Leukemia Group B (CALGB), following this line of reasoning, made a retrospective evaluation of their chemotherapy studies which reveals that the benefits of each new generation of more aggressive chemotherapy are centered particularly in estrogen receptor negative patients. This would suggest that patients with basal-like tumors are those which respond best to strategies such as increases in dose or dosing intensity for anthracyclines, addition of taxanes to anthracycline regimens, or increased dose-density for the drugs administered.

1.14 Justification for the administration of 4 cycles of AC to patients without axillary node involvement.

The first AC studies were carried out at the University of Arizona by Jones, S.B. Adriamycin used in multiple solid tumors began to be investigated in breast cancer showing tumor activity. Based on laboratory study results regarding synergy between doxorubicin, and cyclophosphamide, the first trials were conducted in 1973, in patients with metastatic breast cancer, and were published by S. Jones and S. Salmon (Combination Chemotherapy with Adriamycin and Cyclophosphamide for advanced breast cancer. Jones S.B., Durie B., Salmon S. Cancer 1975, vol. 36. No. 1: 90-97). The use of this combination quickly moved from the metastatic field, to the adjuvant field, both in patients with node involvement (NSABP B15 study) (Fisher B Brown AM, Dimitrov NV, et al. Two months of doxorubicin-cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate, and fluorouracil in positive-node breast cancer patients with tamoxifen-nonresponsive tumors: results from the National Surgical Adjuvant Breast and Bowel

Project B-15. J Clin Oncol 1990; 8:1493-96) without node involvement (Fisher B et al. J Clin Oncol 19 (4): 931-942, 2001).

Cyclophosphamide doses in the AC combination were defined in the NSABP B22 and B25 studies, using doses of 600, 1,200 and up to 2,400 mg/m^2 in 2 to 4 cycles. The ideal dose was defined as 600 mg/m^2 every 3 weeks in relation to efficacy results (same overall survival and disease-free survival).

- Doxorubicin doses were determined via the CALGB 9344 study, in which a standard dose of "A" 60 mg/m^2 was used versus a higher dose of "A" (90 mg/m^2). The efficacy results were similar. For this reason, the ideal AC dose was estimated at AC 60/600.
- The North American cooperative group NSABP compared AC vs CMF in the B15 and B23 studies.
- The B15 study was completed on 2,194 patients with positive nodes, who were recruited between October 1984 and October 1988, and were considered as "non-responsive to tamoxifen". They were randomized to receive 4 cycles of AC at the aforementioned doses, vs 6 cycles of CMF vs 4 cycles of AC, followed by 3 cycles of CMF. The disease-free survival was similar in the three arms (p=0.57) as well as for overall survival (p=0.49). In view of the fact that the standard adjuvant treatment at the time was CMF, the equivalence of AC x 4 cycles was established with CMF x 6 cycles and was the base for the design of multiple subsequent adjuvant studies in patients with positive nodes.
- Given the efficacy equivalence proven by the NSABP study of CMFX6=ACX4 in positive nodes, the B23 study was carried out on 2,008 patients with negative estrogen receptors and negative nodes. They were randomized to receive 4 cycles of AC vs 6 cycles of CMF. The disease-free survival was similar in the 2 arms (p=0.9) as well as for overall survival (p=0.6). These results have been the base for multiple Medical Oncology groups to apply 4 cycles of AC in patients with negative nodes within their treatment standards[106].

The clinical guidelines in certain countries in Latin America establish that patients with negative axillary nodes should receive systemic adjuvant chemotherapy treatment containing 6 cycles of CMF or 4 cycles of AC.

1.15 Justification for the present study

(Neo-) and/or adjuvant treatment studies are the most favorable framework for the evaluation of new proposals for chemotherapy. The subjectivity of evaluation of the response rate observed in the metastatic studies will be replaced by the objectivity of the variables used in these studies (disease-free survival, and finally, overall survival).

As commented, the majority of the relevant studies which are being performed in (neo-) and/or adjuvant treatment at the moment use sequential protocols, with the idea of permitting the administration of the highest possible dose of each drug, while diminishing additional toxicity which may be produced by the simultaneous administration of various chemotherapeutic agents. In this sense, the design of the CIBOMA/2004-01 study is particularly interesting for the following reasons:

- It is directed at a type of tumor with poor outcome, ie. basal-like or triple negative.
  It is postulated that this type of tumor will be best treated by chemotherapy treatments. In addition, in these patients there is no possibility of receiving complementary treatment with hormone therapy or trastuzumab.
- The hypothesis of the possible benefit of a longer chemotherapy treatment (6 cycles of standard (neo-) and/or adjuvant chemotherapy $\pm$ 8 cycles of capecitabine).

- The study drug is capecitabine, which has been shown to be one of the most effective and safest in the treatment of metastatic breast cancer.
- Capecitabine is a drug which is administered orally, and this will allow a long (neo-) and/or adjuvant treatment with chemotherapy for up to 12 months, without affecting the quality of life of the patients treated.

2. STUDY OBJECTIVES AND AIMS

2.1 Principal objective

Compare disease-free survival after maintenance therapy with 8 cycles of capecitabine (X) compared to observation, in patients with operated, hormone receptor and HER2 negative breast cancer who have received standard (neo-) and/or adjuvant chemotherapy.

2.2 Secondary objectives

- Compare 5-year Disease-Free Survival (DFS).
- Compare Overall Survival (OS) between the two above-mentioned groups
- Compare toxicity between the two above-mentioned groups.

2.3 Tertiary objectives

- Determine the treatment effect of capecitabine on the development and duration of amenorrhea in premenopausal women.

- (In selected centers): Study the existence of thymidylate synthase (TS) and methylenetetrahydrofolate reductase (MTHFR) polymorphisms and confirm their relation with toxicity and efficacy of treatment with capecitabine.

3. PATIENT SELECTION

3.1 Inclusion criteria

1. Informed consent must be obtained and documented in writing before any protocol-specific procedures are performed. Information must be included on the expected cooperation of patients in treatment and follow-up, in accordance with the ICH guidelines for Good Clinical Practice. The patient must sign informed consent for sending her tumor sample to the central laboratory. Subsequently, if the patient is eligible, a consent form must be signed for participation in the clinical trial.

2. Histologically-proven breast cancer (histological examination: invasive adenocarcinoma).

3. Patients with ispilateral axillary node involvement. If the sentinel node technique is used, the sentinel node will count as a resected involved node. Patients who can be classified in the following groups (AJCC 2002) will be admitted:
    - pN1a: Metastasis in 1 to 3 axillary nodes.
    - pN2a: Metastasis in 4 to 9 axillary nodes (at least one tumor deposit >0.2 cm).
    - pN3a: Metastasis in 10 or more axillary nodes (at least one tumor deposit >0.2 cm). Patients with a diagnosis of pN3a with infraclavicular node metastasis will **not** be eligible.

Nota: Patients without axillary node involvement (N0), provided the primary tumor is greater than or equal to 1 cm in diameter. If the sentinel node technique is used, lymphadenectomy will not be necessary.

4. Out patient women aged≥ 18 years.
5. Karnofsky performance status ≥80 (ECOG 0,1).

3.2 Exclusion criteria

1. Definitive surgical treatment for operable breast cancer (T1-3, M0) must be mastectomy or breast-conserving surgery. The margins of the sample extracted in definitive surgery must be histologically free of invasive adenocarcinoma and ductal carcinoma *in situ* (DCIS). Lobular carcinoma *in situ* is not considered as a positive margin.
2. (Neo-) and/or adjuvant chemotherapy treatment with anthracyclines and/or taxanes (paclitaxel, docetaxel) of at least 6 clycles has not been received. **NOTE**: For patients without axillary node involvement that have not received at least four cycles of adriamycin and cyclophosphamide as single chemotherapy treatment. The allowed treatments are available in appendix 1. Any other treatment schedule should be approveb by the study medical coordinators.
3. In lymphadenectomy, resection of less than 6 nodes (For patients undergoing biopsy of the sentinel node, if it is positive, will count as a resected).
4. Previous treatment with anthracyclines or taxanes (paclitaxel, docetaxel) for any neoplasm other than the breast cancer being treated.
5. For patients that receive radiation therapy, no more than 8 weeks (2 months) should elapsed between day 1 of last (neo-) and/or adjuvant chemotherapy cycle and the clinical trial entry. For those patients in which the administration of adjuvant radiation therapy, more that 4 weeks (1 month) can be elapsed between the last session and the clinical trial entry. For patients that receive antineoplastic treatment before surgery, the interval between definitive surgical treatment and clinical trial entry is longer than 60 days.
6. Patients with unknown or positive estrogen, progeterone and HER2 receptor tumors.
7. Pregnant or nursing patients. Patients of child-bearing potential must have a negative result in a urine pregnancy test with the 14 days before treatment assignation.
8. Women in a fertile age that do not want to use a reliable and suitable contraceptive method. The postmenopausal women should have been with amenorrhea during at least 6 months to be considered as infertile (see section 6.5).
9. Diagnosis of invasive bilateral breast cancer.
10. Clinically relevant heart failure, such as congestive heart failure or symptomatic coronary artery disease, heart arrhythmia uncontrolled with treatment or history of myocardial infarction during the year before study inclusion, or uncontrolled hypertension.
11. History of significant neurological or psychiatric disease, including psychotic disorders, dementia or attacks which would impede the patient's understanding and giving informed consent, will interfere in compliance of the study drug regimen.
12. Uncontrolled active infection, or other diseases or serious medical pathologies, such as active peptic ulcer, unstable diabetes mellitus.
13. Presence of anomalies in any laboratory parameter, as defined next: (in the previous 14 days to the treatment assigment):
    - Hemoglobin < 10 mg/dl; absolute neutrophil count < 1,5 x 10^9/l; platelet count < 100x 10^9/l.
    - ASAT (SGOT) and ALAT (SGPT) > 2,5 ULN; alkaline phosphatase > 5 ULN; total bilirrubin > 2.0 ULN. Patients with ASAT and/or ALAT values >1.5 x

ULN **associated** with an alkaline phosphatase level >2.5 x ULN will not be selected for the study.

- Severe renal impairment , defined as creatinine clearance below 30 ml/min, according to the Cockroft and Gault formula (see appendix 9) or serum creatinine > 1,5 ULN.

14. Previous or present history of neoplastic disease other than breast cancer, with the exception of:
    - Non-melanoma skin cancer, cervical cancer in situ or other form of tumor curatively treated and without signs of disease for at least 10 years.
    - Ipsilateral ductal carcinoma *in situ* (DCIS) of the breast.
    - Lobular carcinoma *in situ* (LCIS) of the breast.
15. Known hypersensitivity to capecitabine, doxifluridine, fluorouracil or any of its excipients.
16. Patients with lack of upper gastrointestinal tract physical integrity or malabsorption syndrome, or with impossibility to take medication administered orally.
17. Previous treatment with capecitabine or with continuous infusion (> 24 h) with 5-FU, or with other oral fluoropyrimidines, such as eniluracil/5-FU, uracil/tegafur, S1 or emitefur.
18. Blood transfusions or administration of growth factors to help the hematological recovery in the 2 weeks prior to the beginning of the study treatment.
19. Hormone treatment in the 10 days prior to the beginning of the study treatment.
20. Possibility of unexpected severe reaction in combination therapy with fluoropyrimidine, with or without proven dihydropyrimidine dehydrogenase (DPD) deficiency.
21. Allograft transplants of organs that require immunosuppressive therapy.
22. Patients treated with coumarin–derived anticoagulants.
23. Patients treated with sorivudine or chemically related analogs, such as brivudine.
24. Concomitant treatment with other investigational drugs. Participation in another clinical trial with any unmarketed investigational product during the 30 days prior to study inclusion.
25. Concomitant treatment with any other anticancer therapy.
26. Inaccessible patients for the treatment and monitoring. The patients registered on this trial should be treated and monitored in the participating center, that could be the center of the principal investigator or co-investigator.
27. Patients that cannot or do not want to comply with the protocol, considering the total duration of the study.

4. STRATIFICATION AND RANDOMIZATION TO TREATMENT ARM

Participating investigators will be responsible for ensuring that each new patient fulfills the study inclusion criteria, is informed appropriately about the study, reads and understands the patient information sheet and dates and signs the informed consent form for participation in the study.

4.1 Timing of registration and randomization to study

Potentially eligible patients should sign the informed consent form for sending their tumor sample to the central laboratory at the beginning of the standard chemotherapy. Receipt of the sample will coincide with registration of the patient in the study. The tests to be made on the sample are : progesterone, estrogen and HER2 receptor status.

Before the end of treatment with standard chemotherapy, the investigator will receive the response from the central laboratory stating whether the patient can participate in the study.
If the patient is eligible, she must sign a new informed consent form agreeing to participation in the study.

The interval between day 1 of the last cycle of (neo-) and/or adjuvant chemotherapy and randomization to the study must be no more than 8 weeks (2 months). For patients in whom the administration of adjuvant radiation therapy is indicated, the interval between the last session and study entry must be no more than 4 weeks (1 month). For patients that receive antineoplastic treatment before surgery, the interval between definitive surgical treatment and clinical trial entry should be no longer than 60 days. **CLARIFICATION NOTE**: The maximum interval in order to be able to randomize the patient will be taken into account from the last curative treatment that the patient received (surgery, chemotherapy or radiotherapy).

The administrative coordinator of the study must register all selected patients before the beginning of study treatments. Patients who were not registered before administration of the first treatment will not be permitted subsequently to participate in the study.
No more than 8 days must elapse between the date of treatment randomization and the date of the beginning of the first capecitabine maintenance cycle.
The study monitor will notify the investigator by fax and/or electronic mail, within a period of one working day, of the patient study number and treatment arm to which she has been randomized.

4.2 Treatment randomization procedure

The registration forms should be sent by fax or electronic mail to the study administrative coordinator (CIBOMA Central Operations Department)

CIBOMA
Telephone: +34916592870
Fax: +34916510406
ciboma@ciboma.org

Treatment randomization will be carried out when it has been confirmed that the patient was previously registered and is eligible.

4.3 Treatment groups

Patients will be assigned by the randomization system to one of the following groups:

- Arm A: maintenance treatment with capecitabine
- Arm B: observation

4.4 Stratification

Each selected patient will be randomized to a treatment arm, according to a specific block (center, previous chemotherapy treatment with anthracyclines vs. anthracyclines and taxanes, number of involved nodes (0 vs. 1-3 vs. ≥4); and the phenotype (basal vs. triple negative)), to receive X x 8 or observation.

5. PATIENT TREATMENT

Experimental arm: capecitabine 1,000 mg/m^2 twice a day for 14 days, followed by a 7-day rest period, for 8 cycles.

The study treatment is capecitabine (Xeloda®). For study purposes, medication will be defined as the administration of capecitabine in the treatment arm during active treatment. Xeloda® (capecitabine) is a non-cytotoxic fluoropyrimidine carbamate, which, when administered orally, acts as a precursor for the cytotoxic 5-fluorouracil (5-FU). Capecitabine is activated by several enzymatic steps. The enzyme responsible for the final conversion to 5-FU, thymidine phophorylase, is found in tumor tissues, and also in normal tissues, but generally at lower levels.

Control arm: Observation.

5.1 Provision of drug supplies

The pharmaceutical company Roche will provide Xeloda® free of charge, in the form of commercial drug, in sufficient quantities for the treatment of the study patients. The study medication will be delivered by the Pharmacy Departments to the study investigators, who should dispense them to the patients and ensure compliance.

Xeloda® is presented in boxes of film-coated tablets:

- Xeloda® 500 mg: 120 film-coated tablets (12 blister packs containing 10 tablets). The tablets are not fissured, and should not broken.

Xeloda® must not be stored at temperatures above 30ºC. The principal investigators at each center will be provided with a temperature chart which must be completed for the area where the drug is stored at least once a week. Patients will be properly informed about storage conditions for Xeloda®.

5.2 Capecitabine administration

Xeloda® chemotherapy doses will be administered according to body surface area, rounding up or down to the nearest multiple of 500. Please see the table on appendix 10 to determine the proper rounding of the doses according to the body surface area. If variation of the body surface area take place during the study, it will be assumed that the body surface area keep approximately constant, i.e adjustments due to changes in the corporal weight will not be made during the study.

Capecitabine

It will be administered a dose of 1,000 mg/m^2, twice a day (morning and night); equivalent to a dayly dose of 2,000 mg/m^2. A cycle consist on the administration during 14 days, followed by a 7 day rest period. At the discretion of the investigator and patient, the cycles can be calculated in two ways: the first regimen, permit the first dose of each cycle to be administered in the morning of day 1, and the last dose, in the evening of day 14, followed by a 7 day rest period. In this way, the cycle consist on the administration of 28 doses in a period of 14 days. The second regimen permit the first dose of each cycle to be administered in the evening of day 1, and the

last dose in the morning of day 15, followed by a 6.5 day rest period. This results in a total of 28 doses per cycle, in 15 days. Any of these regimens can be used, providing the patient receive 28 doses administered in consecutive days. The patient does not need to follow the same regimen in all the cycles, and can choose to change the regimen from a determined cycle. For example, in the cycle 1, the patient goes to the hospital consulting room and starts the cycle in the evening. Take the last dose in the moning of day 15. In the second cycle, the patient take the first dose in the morning of day 1, and the last one in the evening of day 14. The regimen followed in each cycle should be registered in the CRF. The morning and evening doses must be at least 12 hours distant. The dose must be taken with 200 ml of water during the 30 minutes after the meal. For example, a patient with a body surface area of 1,6 m^2, should receive 1,600 mg. This will be rounded up to administer 4 x 500 mg tablets. The patient will be given enough drug to complete the 28 doses in each cycle of 21 days. 8 cycles of treatment will be administered.

Starting dose in patients with moderate renal impairment
Patients with moderate renal impairment at baseline, defined as creatinine clearance between 30-50 ml/min (calccalculated according to the Cockroft and Gault formula, appendix 9), will start with a dose reduction to 75%, i.e. instead of 1000 mg/m^2 twice a day, they will receive 750 mg/m^2 twice a day.

5.3 Concomitant medication (only applicable to patients of the capecitabine treatment arm)

Phenytoin
A rise in the plasma concentration of phenytoin has been reported, during the concomitant administration with capecitabine. Formal studies of interaction between the two drugs have not been realized. The patients that receive phenytoin concomitantly with capecitabine, Should be regularly monitored to control the phenytoin plasma levels and the associated symthoms.

Halopurinol
Interactions between halopurinol and 5-FU have been observed, with a possible decrease of the 5-FU efficacy. The concomitant administration of halopurinol and capecitabine should be avoided.

Metronidazol
In this study is not permitted the concomitant administration of metronidazol and capecitabine.

Cimetidine
It has been reported that the prior treatment during 4 weeks with cimetidine rise the plasma concentration of fluorouracil, after intravenous and oral administration in 6 patients. The effect was probably due to a combination in the hepatic enzymes inhibition and a reduction in the liver blood flow. This effect has not been observed after the administration of lonely doses of cimetidina to 5 patients or during the 1 week pretreatmentin 6. Caution is advised if an study patient should be concomitantly administered cimetidine and capecitabine.

Hematopoietic growth factors
They can be administered according to the habitual rules in each center, for the treatment of febrile neutropenia. It cannot be administered as primary or secondary profilactic treatment. The administration of hematopoietic groth factors will be stated in the medical record and in the CRF. The administration of groth factors should be interrupted at least 48 hours before the initiation of the next capecitabine cycle.

Laxatives
The administration of drugs with laxative properties should be avoided, due to the risk of diarrhea increase.

Antiacids
The effect of an antiacid that contains alunimium hydroxide and magnesium hydroxide over capecinabine's pharmacokinetics was studied. There were a slight increase in the plasma concentrations of capecitabine and of one metabolite (5´DFCR); there was no effect over the 3 principal metabolites (5´-DFUR, 5-FU y FBAL).

Leucovorin
The concomitant administration with capecitabine can interact with this and increase the 5-FU concentration. As a consequence the toxicity could be increased, that makes necessary the monitoring of these patients.

5.4 Permitted dose adjustments

Toxicities will be classified using the Common Toxicity Criteria of the NCI, version 3.0 (http://ctep.cancer.gov/reporting/ctc.html).

Two possible dose reductions are permitted in the case of hematological toxicity and/or severe non-hematological toxicity as indicated below:

Drug	**From (initial dose)**	**To (reduced dose -75%)**	**To (reduced dose -50%)**
Capecitabine	2000 mg/m^2 (1000 mg/m^2 twice a day)^2	1500 mg/m^2 (750 mg/m^2 twice a day)	1000 mg/m^2 (500 mg/m^2 twice a day)

If the dose is reduced, it must not be increased again at any time in the future. The interruptions in the capecitabine treatment will be considered as lost treatment days, and the initial treatment plan will be maintained. Doses not administered due to toxicity will not be substituted or re-established (see appendix 9).

If a patient experiments several toxicities of different grade and intesity at the same time, the greater possible dose reduction will be applied.

If the value of the creatinine clearance decreases during the study to a value $\leq$ 50 ml/min, due to an increase in the serum creatinine or a decrease in the body weight, this change is not a reason, in itselft, for a dose reduction. Dose reductions during treatment may be based in adverse events.

Regarding the adverse events that are apparent at baseline, the dose modifications will be applied according to the corresponding change in the toxicity gradation, If the investigator considers it suitable. For example if a patient presents grade 1 asteny in the baseline, that rises to grade 2 during treatment, the different to be considered is the rise to grade 1 in toxicity, to make the dose modifications.

In the case of toxicities that the investigator considers improbable to become serious or life threatening (for example, alopecia, taste alterations), the drug dose must not be reduced neither discontinued. Dose reductions or interruptions are neither required in case of anemia (not hemolitic), if it is satisfactory treated by transfusions.

In case of grade 1 toxicity, or alopecia, the treatment will continue at the initial dose without interruptions.

The following criteria will be applied for the reduction or delay of capecitabine dosing due to toxicity:

Dose reduction schedule for capecitabine monotherapy- not hematological toxicity		
	Grades 2 and 3	Grade 4
-1st appearance	Discontinue until the remission to grade 0-1; continue the capecitabine administration to 75% of the initiation dose, adding prophylaxis if possible.	Discontinue indefinitely or hold until return to grade 0-1 if the doctor considers that it is the patient's best interest to continue, reduce dose to 50% of that planned.
-2nd appearance	Discontinue until the remission to grade 0-1; continue the capecitabine administration to 50% of the initiation dose.	
-3rd appearance	Discontinue treatment indefinitely, unless the doctor considers that it is the patient's best interest to continue.	

Capecitabine can cuse diarrhea, serious in occasions. In patients that receive capecitabine monotherapy, the median of time until the appearance of grade 2-4 diarrhea is 31 days, and the median duration of the grade 3 or 4 diarrheas was of 4.5 days. Patients with serious diarrhea should be carefully monitored, and in case of dehydratation the administration of fluids and electrolytes will be assured. In case of grade 2, 3 or 4 diarrhea the administration of capecitabine may inmediatly discontinue, until its solving or decrease to grade 1. The next episodes of grade 2 or superior diarrhea, require a capecitabine dose reduction, as stated in the table above. Treatment with standard antidiarrheal drugs, such as loperamide, sould be initiated as soon as possible. The capecitabine treatment should not be reinitiated until diarrhea has been reduced to grade 0-1, having administered the last loperamide dose at least 24 hours before.

Nausea /Vomiting grade ≥ 2

Patients should be provided with antiemetic drugs for auto-administration in case of nausea and vomiting if these happen at home. Metoclopramide +/- antagonist 5-HT3 is recommended for the nausea inducted by pecitabine. The proper prophylactic and secondary therapies will be initiated if nausea and vomiting have occured. If nausea and vomiting are recurrent, despite the prophylaxis, a dose modification will be made according to that stated in the corresponding chart.

Skin toxicity: grade 2/3 hand-foot syndrome

The hand-foot syndrome (Palmar-plantar erythrodysesthesia or chemotherapy inducted erythema) are considered as skin toxicity.

If a grade 2 or 3 hand-foot syndrome occurs, the capecitabine administration must be discontinued until the solving of reduction to grade ≤ 1. the later dose reductions are summarized in this section.

The hand-foot syndrome must be symptomatically treated (for example, it is recommended the use of emollients). A possible profit of the use of B6 vitamin p pyridoxine[73,74], and is permitted as symptomatic treatment or secundary profilaxis of the hand-foot syndrome.

5.4.1 Warnings and cautions

The capacitabine is administered with occasional assistance, and in certain cases, adverse effects as diarrhea can rapidly become into serious events. In case a patient experiences any toxicity between two arranged visits, the patient must be instructed in calling the doctor as soon as possible, so that he or she can explain the patient about the possible treatments. It is important to instruct the patients in discontinuing capecitabine treatment as soon as the grade 2 toxicities of the most frequent events, such as diarrhea, hand-foot syndrome or stomatitis appear.

Capecitabine is contraindicated in patients with severe renal impairment (creatinine clearance below 30 ml/min (appendix 8)). Patients with severe renal impairment are not eligible for this project. If the severe renal impairment occurs during the study treatment, it will be immediately discontinued. It has been informed that in patients treated with capecitabine, and that presented moderate renal impairment (creatinine clearance 30-59 ml/mim), the incidence of grade 3 or 4 adverse event increases. If a patient with moderate renal impairment is included in the study, the treatment will initiate with the corresponding dose to 75%. Patients with mild renal impairment (creatinine clearance of 51-80 ml/mim), no adjustment of the baseline dose will be made, but the appearance of grade 2, 3 or 4 toxicities will be monitored to make the dose adjustment established by protocol.

Events have been reported of cardiotoxicity associated with fluorinated pyrimidines (including capecitabine), that include the myocardial infarction, angina, dysrhythmias, cardiogenic shock, sudden death and electrocardiograph changes. These adverse events may be more common in patients with a prior history of coronary artery disease.

In patients with elevation in bilirubin of > 3 ULN, or with elevations in hepatic transaminase enzymes of > 2.5 ULN, treatment related, the administration of capecitabine will be discontinued. It will be continued when the bilirubin levels reach $\leq$3.0 ULN, or hepatic transaminase enzymes reach < 2.5 ULN.
Patients older than 80 years have more risk to experiment grade 3 or 4 toxicity, particularly diarrhea, nausea, hand-foot syndrome and vomiting.

5.5 Drug accountability

The person responsible for dispensing the medication must maintain an appropriate record of the drug administered during the study. This record includes the date in which the study medication was received, and the dates on which it was dispensed to the patient, the number of tablets administered in each visit and the number of tablets returned in the next patient visit.

5.6 Standard (neo-) and/or adjuvant chemotherapy

Standard (neo-) and/or adjuvant chemotherapy before randomization will consist of 6 cycles of combinations of anthracyclines with other antineoplastic agents, or anthracyclines and taxanes, or a minimum of four cycles of adriamycin and cyclophosphamide as single chemotherapy treatment for patients without axillary node involvement. Appendix 1 sets out the table of prior (neo-) and/or adjuvant chemotherapy regimens permitted in this study which the patients must have completed to be eligible for the CIBOMA/2004-01 study.

6. EVALUATION OF EFFICACY

6.1 Primary endpoint: evaluation of treatment efficacy

All randomized patients will be included in an intent-to-treat analysis.

Primary efficacy evaluation will consist of the comparison of disease-free survival (DFS) between the treatment groups. Disease-free survival is defined as the interval between the date of randomization to the date of local, regional or metastatic recurrence or the date of appearance of a second primary cancer (except squamous cell or basal cell skin cancer, cervical carcinoma *in situ* or lobular carcinoma *in situ* of the breast) or death from all causes, whichever occurs first.

If study treatment is discontinued (for whatever reason), disease-free survival and overall survival of the patient will analyzed in the intent-to-treat analysis.

Variables which will be used to measure treatment efficacy are:

6.1.1 Objective Recurrence

Any clinical or radiological sign of tumor recurrence, including in the central nervous system. Histological or cytological proof of failure must be obtained, if possible. The appearance of any sign of malignant disease must be detailed in the diagrams. The patient will be followed up for survival.

6.1.2 Local Recurrence

Defined as recurrence of the tumor in the surgical scar in the breast, ipsilateral breast (in the case of breast-conserving surgery) or signs of tumor in the ipsilateral anterior chest wall (in the case of mastectomy) or in the skin or soft tissues within the local area. *Confirmatory histology or cytology will preferably be performed.*

6.1.3 Regional Recurrence

Defined as recurrence of tumor in the axillary scar, ipsilateral nodal areas (axillary, supraclavicular, internal mammary and infraclavicular nodes), or in the skin or soft tissues within the regional area. *Confirmatory histology or cytology will preferably be performed.*

6.1.4 Distant Recurrence

Defined as signs of tumor outside the locoregional area, as defined above.

This includes the following:

1) lymph nodes not included in the above-mentioned areas (i.e. contralateral axillary, paratracheal, etc.)
2) skin, not including the areas defined above.
3) liver
4) lung
5) bones
6) central nervous system
7) contralateral breast
8) other localizations not previously defined.

Confirmatory histology or cytology will preferably be performed, especially for solitary lesions. Positive bone scans must correlate with the bone X-ray.

Multiple pulmonary lesions in the chest X-ray, multiple hepatic lesions in the hepatic ultrasound or CT, multiple lytic or blastic bone lesions or multiple foci of increased uptake on bone scan will be acceptable without pathological correlation.

6.1.5 Second primary tumor

This is defined as any other histopathologically documented tumor, including second primary invasive breast cancer in the ipsilateral or contralateral breast, except for non-melanoma skin cancer, cervical carcinoma *in situ* and breast carcinoma *in situ* (LCIS/DCIS).

6.2 Secondary endpoint: 5-year Disease-Free Survival

6.3 Secondary endpoint: Overall 5-year survival

Survival will be determined from the date of randomization to the date of death for whatever reason.

6.4 Secondary endpoint: Evaluation of treatment safety

6.4.1 Clinical Safety

The following tests will be performed before therapy and on specific days during therapy:

- Complete history of neoplastic and non-neoplastic diseases, including cardiac history.
- Complete clinical examination, vital signs (blood pressure, pulse, temperature), height, weight, evaluation of any residual toxicity due to any previous treatment, evaluation of Karnofsky performance status.
- Chest X-ray
- Adverse events: a regular evaluation of each patient will be carried out to determine possible adverse events according to the NCI-CTC scale (version 3.0).
- Laboratory tests: the following tests will be carried out before and on specific days during therapy:
    - Hematology: White cell count, neutrophils and platelets, hemoglobin
    - Biochemistry: Usual hepatic and renal function tests (creatinine or clearance)
- In case of bone pain, raised alkaline phosphatase or elevated tumor markers, a bone scan must be performed. In any case, a baseline bone scan is recommended for use in possible subsequent follow-ups.

Toxicities which cannot be classified using the NCI-Common Toxicity Criteria will be defined as follows:

a) mild (asymptomatic)
b) moderate (symptomatic, but does not interfere significantly with activity)
c) severe (significantly interferes with activity)
d) life-threatening

6.5 Tertiary endpoint (in specific patient populations)

A blood sample will be obtained from each patient before beginning treatment, for the prospective testing of the effect of TS and MTHFR polymorphisms associated with the efficacy and toxicity of capecitabine administration. The sample will be processed to obtain white cell series and plasma.

In the laboratory, white blood cells will be extracted from the sample and DNA will be isolated from these cells using the QIAamp mini-kit for DNA purification from Qiagen. The region of

the TS promoter will be amplified by PCR using primers already described in the literature. The products of the reaction will be resolved in 1% agarose gel. The PCR products of different lengths will be sequenced and the fragments representative of 2R (220 pb) and 3R (248 pb) will be identified. These products will be used as size markers and will be included as controls in subsequent studies.
The 3R products will be sequenced for polymorphisms described in the literature. The samples identified as 3R alleles will be cloned by T-vector cloning (Invitrogen) and the insert will be sequenced with vector primers, using sequencing kits and a capillary electrophoresis-based genetic analyzer from Applied Biosystems. The GeneTools program from Biotool will be used to analyze sequencing data.
The genotype of the patients will be found in the 6 pb region of the 3'UTR end for polymorphisms, according to the method of Ulrich. The following primers will be used. 5´CAAATCTGAGGAGCTGAGT3´and 5´CAGATAAGTGGCAGTACAGA3´. PCR conditions will be the following: 1 cycle at 94ºC for 5 minutes; 30 cycles at 94ºC for 30 seconds; 57ºC for 50 seconds; 74ºC for 45 seconds, and 1 cycle of 5 minutes a 72ºC. The fragments will be digested with DraI and will be separated using 3% agarose gel. The 6 pb deletion produces 152 pb fragment and wild-types produce fragments of 70 and 88 pb.

To determine MTHFR genotypes, single nucleotide polymorphisms in the nucleotide positions 677 T/C and 1298 A/C will be analyzed with the databases for the identification of single nucleotide polymorphisms rs1801133 and rs1801131 (Genbank ref. mRNA NM 005957), respectively, using the PSQ HSA96 pyrosequencer. The following primers will be used:

- C677T forward primer: 5´CTAGGAAGGTGCAAGATCAGAG;
- C677T reverse primer: 5´Biotin-GTGTCAGCCTCAAAGAAAAG
- C677T sequencing primer: GGTGTCTGCGGGA.
- A1298C forward primer: 5´AACTCACTTTGTGACCATTCC
- A1298C reverse primer: 5´Biotin-GGGGAGCTGAAGGACTACTA
- A1298C sequencing primer: 5´AAGACTTCAAAGACACT

The simple biotinilated DNA chain of MTHFR polymorphisms and nearby regions will be generated using standard PCR techniques and by sequencing the genotypes using the pyrosequencer according to the instructions of the manufacturer. The PCR primers will be designed using the Primer3 program (Whitehead) and the sequencing primers will be designed using the Pyrosequencing Inc. program. The synthesis of all primers will be commissioned from Qiagen Genomics.

Blood levels of thymine and uracil will be evaluated for the determination of DPD deficiencies. Proteins will be eliminated from the plasma using trichloroacetic acid, and it will be neutralized with tri-n-octylamine in 1,1,2-trichlorofluoroethane. Nucleosides and bases will be separated by reverse phase liquid chromatography using a Symmetry C-18 column, according to methods already described.

6.6 Tertiary endpoint

The effect of capecitabine treatment on the development and duration of amenorrhea in women who are premenopausal pre-treatment will be studied using the questionnaire of Dr Cobleigh of the Rush Presbyterian St Luke Hospital, Chicago (Appendix 8). The menopausal status of each patient will be defined pre-inclusion, according to the following definitions:

a) Premenopausal

- No prior hysterectomy; menstruation during the 6 months prior to randomization
- Prior hysterectomy; and/or <40 years of age, or ≥40 years of age with premenopausal LH and FSH values

b) Postmenopausal/Perimenopausal

- No prior hysterectomy; NO menstruation during the 6 months prior to randomization

- Prior hysterectomy; and/or >55 years of age or ≤55 years of age with postmenopausal LH and FSH values.

Menstrual status questionnaires will be distributed to patients classed as premenopausal in the randomization form.

7. STUDY EVALUATIONS

Complete diagnostic disease-staging procedures will be carried out in the months prior to study inclusion. If staging carried out before the start of standard chemotherapy is available, it will not be necessary to repeat the tests. All patients must have a bilateral mammography, chest X-ray (PA and lateral), abdominal ultrasound and/or thoracic-abdominal CT. In case of bone pain and/or elevated alkaline phosphatase figures, a bone scan must be performed: in any case, a baseline bone scan is recommended in all patients included in the study. Other tests may be performed as clinically indicated.

7.1 Time and events schedule

(x: obligatory; ♦: if clinically indicated; rd: randomization; CT: chemotherapy; Arm A: capecitabine; Arm B: observation)

0= Rd day	Pre-rd	Arm A				Arm B	
Cycle day		During CT	During QT	Years 1-2	Years 3-5	Years 1-2	Years 3-5
Frequency		Day 1 or day -1 of each cycle	Day 1 or day -1 of cycle 4	Every 3 months	Every 6 months	Every 3 months	Every 6 months
Informed consent	X						
History	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X
Blood tests							
Hematology	X	X	X		♦	♦	
Biochemistry	X		♦		♦	♦	
Pharmacogenetics**	X						
Blood or urine test							
Pregnancy test	X						
Tumor evaluation							
Mammography***	X				♦	♦	
Chest X-ray***	X				♦	♦	
Abdominal ultrasound or CT***	X				♦	♦	
Bone scan***	♦						
Tumor tissue evaluations							
Hormone receptor status*	X						
HER2 status*	X						
Other							
Adverse events		X	X	X	X	X	X
Evaluation of amenorrhea (premenopausal women only)	X	X	X	X	X	X	X
Menopausal status		X	X	X	X	X	X

*To be carried out in the designated central laboratory
**In selected patients only.
*** If the extension study is available before starting standard chemotherapy, repetition of the tests will not be necessary.

Laboratory tests will be carried out, as far as possible, in the same laboratory throughout the study.

Every attempt will be made to use the same radiological examinations from the baseline evaluation to follow-up.

7.2 Withdrawal criteria and planned analysis for withdrawals and dropouts

Patients may be withdrawn from the study for the following reasons:

a) Disease recurrence, appearance of a second primary cancer (except for non melanoma skin cancer treated with curative intention or cervical carcinoma *in situ*), death or administration of other systemic antineoplastic treatment other than the study drug.
b) Unacceptable toxicity according to the criteria of the investigator
c) Capecitabine dose interruption for more than 3 weeks.
d) Intercurrent disease or other reasons which, in the opinion of the investigator, significantly affect the evaluation of the clinical status and which require discontinuation of the treatment.
e) Patient's request to withdraw
f) Decision of the sponsor (for example, protocol violation)

The reason and date of treatment discontinuation will be documented for all patients in the case report form (e.g., end of study, adverse event, lost to follow-up).
A regular follow-up will be carried out on patients who leave the study for any reason other than to receive treatment for disease recurrence or a second primary cancer.

7.3 Treatment after discontinuation of study treatment

If the patients withdraw from the study due to disease progression, the administration of additional treatment will depend on the investigator's criteria.
The administration of any other antitumor therapy (surgery, chemotherapy, immunotherapy) before the documentation of tumor progression is not permitted. If this is not possible, it will be considered that the patient had progressed on the date on which the new antitumor therapy was initiated and the case will be analyzed in the category of failure for the purposes of the calculation of disease-free survival.

7.4 Adverse events

Definition of an Adverse Event

The investigator will instruct the patients to report the occurrence of any adverse event. *An adverse event is any untoward effect associated with the use of a medication, whether considered related to the medication or not, including any side effect, injury, toxicity or sensitivity reactions. This also includes any untoward clinical or laboratory changes not normally present in the patient.*

- Serious Adverse Event (SAE)

A serious adverse event is one that has a significant risk, a contraindication, a secondary effect or a precaution. A serious advert event is considered one that at any dose, comply with at least one of the following criteria:

- Results in death (if this is not integrated in the of efficacy effect).
- Is life-threatening (means that the patient had an immediate risk of dying because of the event in the moment in which it happened. It does not include the event that, if it had been stronger, it would have caused death).

- Requires patient hospitalization prolongation (by hospitalation it means an unexpected hospital entering)
- Results in persistent or significant disability/incapacity
- Results in a congenital abnormality or birth defect
- Is considered a clinically important event or require an intervention to prevent any of the above mentioned endings.
  Clinically important events are those which may not be immediately life-threatening but are clearly clinically significant and relevant. The patient may be jeopardized or may require intervention to prevent a serious outcome. Cancer or overdose or drug abuse will normally be considered serious.
  Medical and scientific judgement should be made in order to decide the notification to the sponsor in other situations, such as medical events that may not be immediately life-threatening, or cause death or hospitalization, but that jeopardize the patient or may require an intervention to prevent the previous list endings. Generally, these situations are also considered serious adverse events. Examples of these events are the urgent service attendance or the home attendance, due to bronquiospasm, blood dyscrasias or convulsions that do not cause hospitalization, or development of drug addiction or drug abuse.

  An unexpected adverse event is the one that due to its nature or seriousness is not indicated in the product specifications.

  The investigator may initially evaluate the causal relation. There are two possibilities:
  - No (it is not related with the study drug).
  - Yes (remote, possible, probable or definitive relation with the study drug).

The term "severe" is used as an intensifier, although a severe adverse event has not necessary to be serious. For example, nausea of several hours duration can be classified as severe, but are not clinically serious.

All serious adverse events which occur during the study treatment period or in the 30 days after administration of a study drug must be reported in the manner described below. For any late onset SAE (occurring after this 30-day period) which is possibly or probably related to study treatment, the same reporting procedure must be followed.

The progression of any underlying disease in the patient which results in one of the above events must be reported as a serious (but expected) adverse event which (a) is not related to the study medication or (b) is caused by the failure of the anticipated therapeutic effect of the study medication.

If the adverse event is serious, it should be reported to the study administrative coordinator, in the Central Operations Department of CIBOMA *within one working day* from the time in which the investigator is aware of the event, using the adverse events report form provided in the investigator file:

CIBOMA
Telephone: +34 9 6512870
Fax: +34 916510406

The Scientific Office of CIBOMA will report all serious and unexpected adverse events possibly related to the study treatments which occur in our country to the person responsible for drug safety in the pharmaceutical companies/manufacturers of the study drugs, to the health

authorities in the participating countries, to the Clinical Trials Committees (IRBs) involved in the trial and the investigators, within the time limits established by the applicable Spanish regulations.

The premature termination of the study and therapeutic measures which should be adopted will depend on the investigator's criteria. A full explanation will be provided in the premature study termination section of the case report form page. The investigator will carry out a follow-up of all adverse events, whether serious or not, until they have satisfactorily resolved.

The investigator and persons responsible for patient care must instigate any complementary studies of serious adverse events, on the basis of what, in their clinical opinion, are the possible causative factors. This procedure may include the need to obtain the additional opinion of an expert in the field of the adverse event. If a patient dies, all post-mortem findings must be provided to CIBOMA, including histopathology.

All other adverse events will be collected in the case report form during the course of the study.

The investigator must accept these responsibilities as set out in the terms of the protocol.

Deaths during the Study

Any death which occurs during the active treatment phase of the study or during the 30 days following the last administration of study drug, whether related to the study drug(s) or not, must be reported within 24 hours to the Scientific Office of CIBOMA. After the 30 days following the last administration of study drug, it will only be necessary to report as serious adverse events any deaths which are considered as being possibly related to the study drug. All deaths must be recorded in the death report section of the CRF, no matter what their cause.

The cause of death (cancer-related, treatment-related, not cancer-related nor treatment-related) must be documented.

8. DATA COLLECTION AND PROCESSING

8.1 Monitoring, Auditing and Inspection

This study will be monitored in accordance with Good Clinical Practice Guidelines (GCP), by regular visits to the study center and telephone conversations between the study monitor and the investigator. During the center visits, the monitor should review the patient source data, the drug accountability forms and the study documentation files. In addition, the study monitor must observe the study procedures and discuss any problems with the investigator. During the study, the health authorities may carry out an inspection of the center. The investigator will provide direct access to the source data/documents for study monitoring, audits and review by the Clinical Research Ethics Committee and the health authorities.

8.2 Data recording

CIBOMA will provide Case Report Forms (CRFs) in electronic format, in which the investigators can record patient study data. CIBOMA has a Secure Server (SSL certificate) exclusively dedicated to the recording of electronic case report form data, set up with the bandwidth necessary for obtaining a good connection. CIBOMA guarantees that this server and the programming of the case report forms will be maintained throughout the study.
The electronic case report form will include an electronic signature for authorized access, a programming module for traceability of data recorded, changes made, and automatic blockage following the time established after recording.
Data recording on the CRF may be direct (access in real time from the point of origin) or deferred, for which a system for replication and transmission on connection will be programmed.
The CRF will be designed with a filter specification developed by the Data Management department of CIBOMA. The aim of the filters is to check data omissions, corrections, discrepancies and clarifications. The filters are applied to increase precision both in data entry and in the Case Report Forms.

After data entry and verification, the validation program will be run, after which the queries will be printed for dispatch to the investigator.

The monitor must verify the CRFs, comparing them with the source data (clinical records, specialist medical reports, etc). Printed copies of all CRFs from patients included in the study in each participating center will be provided on completion of the study. The principal investigator should provide a certificate of conformity, indicating agreement with the contents of each CRF, which will be kept in the central study file.

The design of the Case Report Form for this study will be electronic and will comply with the international ISO guidelines for data protection in “computer networks” and other information-flow systems, and the IEEE for the standardization of verification, validation and documentation plans for the programming used to create the Electronic Case Report Form. The FDA guidelines for records and electronic signatures, GMP and GLP, and quality systems guidelines will also be complied with.

8.3 Identification of data which can be recorded directly onto the CRF and as such will be considered as source documents

The following data may be transcribed directly onto the CRF:

- Patient weight before each cycle (except for first visit, in which the body surface area must be calculated)
- Performance status in each visit (except in the visit prior to randomization-inclusion criteria)

9. STATISTICS

9.1 Study populations

The primary efficacy analysis will be carried out on the **intent-to-treat population (ITT)**, which is defined as the population of all randomized patients, analyzed within the treatment group to which they were assigned. Randomized patients who did not receive chemotherapy will be analyzed with their randomization group. The ITT analysis will be carried out for DFS and OS. In addition, the survival analyses will be carried out on the **population of selected patients**, made up of those patients in whom no major protocol violation occurred.

Toxicity and quality of life analyses will be carried out on all study patients who have received at least one treatment cycle, or who have completed the observation period equivalent to one cycle.

9.2 Statistical methods

Continuous random variables will be expressed as measures of central tendency: mean and/or median and measures of dispersion: standard deviation and range. Quantitative variables will be expressed as percentages. The 95% confidence interval of each of the estimates carried out will be calculated, where possible.

The Kaplan-Meier limit-product method will be used to estimate DFS and OS. The comparison of these 2 parameters between the two treatment groups will be carried out using the Log Rank test. All tests of the hypotheses will be two-tailed. 95% confidence intervals will be calculated for the medians of survival using the Simon method.

In addition, a Cox's multiple regression analysis will be performed for DFS and OS, with the aim of adjusting the treatment comparison for the principal prognostic factors. These factors include age, menopausal status, type of surgery, histopathological findings, and tumor size. The Wald test will be used to establish the prognostic importance of each covariant. The covariants which appear unbalanced in the baseline values will possibly be added to the Cox model. The subgroup analysis will be carried out only if the level of statistical significance in the preliminary test is very high ($p<0.01$).

For the statistical analysis, a center will correspond to a participating hospital. It is expected that by the end of the study there will be a large number of centers with few patients included in each. Consequently, it is not planned to include center effect in the statistical analysis.

Nevertheless, in case a considerable difference in recruitment is produced some centers, a comparison is planned of the consistency of results between these centers and the overall study results, in terms of principal baseline characteristics and primary objective.

9.3 Safety evaluation

The Common Toxicity Criteria of the National Cancer Institute (NCI-CTC) and the corresponding classification system will be used to classify adverse events recorded in the CRF. For adverse events not classified in the NCI-CTC, the COSTART (FDA 1989) classification will be used (severity: 1=mild; 2=moderate; 3=serious; 4=life-threatening).

Adverse events will be compared using the Cochran-Mantel-Haenszel test, the Pearson χ^2 test or the Fisher exact test, all two-tailed. In view of the large number of statistical tests planned, p-

values will not be interpreted in the usual way; instead they will be used as "signaling tool" to highlight differences which justify additional attention.

9.4 Determination of sample size

The following data were obtained from the database of the "El Alamo" project. One thousand six hundred and twenty-seven (1,627) in total were considered during the years 1990 to 1997. The population is formed of patients with operable breast cancer, with surgery, positive nodes, and negative hormone receptors, or negative nodes, negative hormone receptors and T2-3 tumors.
Assuming an exponential distribution, the aim is to detect an increase from 64.72% to 73.7% in 5-year disease-free survival (DFS), corresponding to a hazard ratio of 0.701 and a risk reduction of approximately 30%, with a power of 80% at a two-sided level of 0.05, considering 4 years of recruitment and 3 of follow-up. We would need to see a total of 255 events for DFS, 834 patients without considering drop-outs.

Considering a drop-out rate of 5% post-randomization, the final sample size will be 876 patients, 438 per treatment arm.

The sample size calculation has been completed using the EAST statistical software, version 5.2.

9.5 Statistical analysis of TS and MTHFR pharmacogenetic data

9.5.1 Hypothesis
It is estimated that a 40% difference in overall grade 3/4 toxicity will be detected between 2R/2R patients and 3R/3R patients, the latter being those who present less toxicity. It is expected that TT homozygous individuals will have higher toxicity with capecitabine and that that clinical significance will be found when patients are stratified for polymorphisms in the TS promoter region.

9.5.2 Calculation of sample size
Sample size requirements are based on the principal study endpoint, not on pharmacogenetic variables. However, the study will have statistical power to determine if polymorphisms in the TS promoter are associated with differences in the rate of aggregated toxicity. In a retrospective study in patients with metastatic colon cancer, toxicity rates of 63% and 27% were associated with 2R/2R and 3R/3R polymorphisms, respectively[53]. The most common alleles in Caucasians and Asians are 2R and 3R. In contrast, the alleles 4R, 5R and 9R are more frequent in those of African descent[52]. The expected frequency of allele tandem repeats 3R and 2R are 0.6 and 0.4, respectively, and the assumed genotype frequencies for 2R/2R, 2R/3R and 3R/3R are 0.16, 0.48 and 0.36, respectively. A minimum of 17 2R/2R and 37 3R/3R patients will be required to detect a difference in aggregated toxicity between 2R/2R (60%) and 3R/3R (20%), using the two-tailed χ^2 test with alfa=0.05 and beta=0.20.

The frequency of MTHFR homozygous individuals in the Caucasian population is at least 10%[64]. The study of Cohen et al[63] allows the sample size necessary for studying the effects of MTHFR on the efficacy of capecitabine to be estimated. Response rates of 58% were observed in individuals with CC and CT genotypes compared to a 100% response rate in individuals with TT genotype. These estimations of response and allele frequency suggest that a sample size of at least 80 patients is required for alfa=0.05 and beta=0.08.

9.5.3 Statistical analysis

Patients with DPD deficiencies will be excluded from the analysis, to avoid interferences in the interpretation of data for TS genotype and toxicity. Patients who present toxicity during capecitabine treatment will be classed as i) grade 1/2, or ii) grade 3/4. The principal analysis will compare overall grade 3/4 toxicity rates among the three TS genotypes, using the χ^2 test, followed by a descriptive analysis of specific toxicities (diarrhea, mucositis, neutropenia and palmar-plantar ethryrodysesthesia). Models of association of toxicity and TS and MTHFR genotypes will be made using logistic regression tests.

With regard to survival analysis, Kaplan-Meier curves will be calculated for each TS genotype and compared with curves generated for the log-rank test, with levels of significance established at $p<0.05$ for the two-tailed analysis. No adjustments are planned for multiple analyses. The following comparisons will be made:

i) Only in patients receiving capecitabine: disease-free survival will be compared between individuals with 2R/2R genotype and those with different genotypes.

ii) In patients not receiving capecitabine: disease-free survival will be compared between individuals with 2R/2R genotype and those with different genotypes, to rule out genotype acting as a prognostic factor.

iii) Disease-free survival will be compared between individuals with 2R/2R genotype treated with capecitabine, and those with the same genotype not treated with capecitabine.

iv) Disease-free survival will be compared between individuals with 2R/3R genotype treated with capecitabine, and those with the same genotype not treated with capecitabine.

v) Disease-free survival will be compared between individuals with 3R/3R genotype treated with capecitabine, and those with the same genotype not treated with capecitabine.

10. PRACTICAL CONSIDERATIONS

10.1 Independent Data Monitoring Committee (IDMC)

10.1.1 Composition and Mission of IDMC

The Independent Data Monitoring Committee (IDMC) will be composed of three oncologists and an expert in statistics. These members will be independent from the clinical study and will familiar with the methodology of oncology studies. They must be aware of the danger of drawing conclusions from immature data, and will agree with the study design and objectives of the protocol.

The mission of the IDMC will be the ethical performance of this clinical study and protection of the safety interests of the patients participating in this study. The committee will ensure the feasibility and progress of the clinical study. The IDMC will be responsible for the review of both study efficacy and study safety.

10.1.2 IDMC meetings

The IDMC will meet annually, unless any important circumstance should occur which requires a meeting of its members. The committee will meet annually to review the incidence and severity of each of the serious and non-serious events. If a serious unexpected event or incident is reported before a programmed meeting, a meeting will be called immediately to evaluate and ensure the safety of the study patients. This may also include data from other studies with the same product that is being administered in this study, or new efficacy data from other relevant studies, the results of which may affect the study CIBOMA/2004-01 in some way. The procedures and operations of the IDMC will be recorded in writing, and minutes will be produced for all meetings.

10.1.3 IDMC recommendations

At the end of each IDMC meeting, the recommendation will be stated in writing whether to modify the study (including reasons), publish the results of the interim analysis (including reasons) or continue with the study without modifications.

10.2 Study budget

Part of the budget assigned to this study is available for the payment of investigator's fees. The participating hospitals will be paid for the indirect costs generated due to the realization of the study. Extraordinary direct costs are not expected, since the diagnostic tests are standard for this type of patient. The detailed budget will be provided in a separate document.

10.3 Third-party insurance policy

CIBOMA has taken out such an insurance policy with the company HDI HANNOVER INTERNACIONAL (ESPAÑA) SEGUROS Y REASEGUROS, S.A. to cover all subjects participating in the study for any possible damage they may suffer as a result of their participation in the trial. A certificate of the study insurance policy specific to each center will be sent to the Clinical Research Ethics Committees involved.

10.4 Study archives

The investigator must keep copies of all relevant information for a period of at least 15 years from study termination. CIBOMA will provide the investigator with files for all the necessary study documents. CIBOMA will also provide a list of all documentation which must be kept.

10.5 Publication policy

CIBOMA commits to publishing the study results and will respect the rights of the investigators and centers participating in the study.

11. ETHICAL CONSIDERATIONS

11.1 Declaration of Helsinki

This study will be carried out in accordance with the Declaration of Helsinki (amended in Edinburgh, 2000), as described in Appendix 1, following the Good Clinical Practice Guidelines of the International Conference on Harmonization (GCP/ICH) and in compliance with the legislations applicable in each country.

11.2 Informed Consent (Appendix 4)

The patient will first sign an informed consent authorizing the dispatch of her tumor sample to the designated central laboratory.
Before screening, the patient will be informed about the nature of the study medication and she will be provided with relevant information about the proposed aims, the possible benefits and possible adverse experiences. The procedures and the possible risks to which the patient may be exposed will be explained.

The patient and the investigator will then read and sign the informed consent. A signed and dated copy of the informed consent will be provided to the patient. The patient may withdraw from the study at any time, without affecting her future medical care. A note verifying signed informed consent will be entered in the study patient's CRD.

11.3 Confidentiality

The investigator will be responsible for maintaining the information required on each patient (e.g. initials of name, address, telephone number, social security number and study identity), in a way in which the health authorities or CIBOMA can access this information, if required, while maintaining confidential the name of the patient. This information must be kept confidential for the legally stipulated period, as required by Spanish legislation.

11.4 Clinical Research Ethics Committee (CREC)

A duly constituted Ethics Committee will review the final approved protocol and informed consent. The decision of the Ethics Committee regarding the realization of the study will be submitted in writing to CIBOMA and to the investigator.

CIBOMA and/or the investigator agree to present to the CREC the necessary reports on study progress, and to report any serious adverse events, life-threatening events or deaths. The investigator will also inform the CREC of any cases of serious adverse events (provided by CIBOMA) reported in other clinical studies carried out with the study drug. The investigator must inform the CREC of the termination of the study.

11.5 Protocol Modifications

A significant protocol amendment is any modification which may affect the realization of the study, the potential benefits to the patient or which may affect patient safety, including changes in the study objectives, study design, patient population, sample size, study procedures or significant administrative changes. The amendment will be authorized by CIBOMA and the investigator and approved by the Ethics Committee and the health authorities before implementation, in accordance with local legislation.

A non-significant protocol modification consists of minor corrections and/or clarifications which do not affect the manner in which the study is performed. These non-significant amendments will be authorized by CIBOMA and the investigator and will be documented in a

memorandum. Non-significant modifications will be reported to the CREC and to the health authorities, in accordance with local legislation.

11.6 Patient Identification

In the initial visit, the initials and date of birth of patients screened for the study will be recorded chronologically in the investigator's file. If any patient is excluded from participation in the study, the reason for this must be documented in the space provided.

A study number will be assigned to each patient at the time of registration. The Patient Assignation Number and her initials must be recorded on the Case Report Form.

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APPENDIX 1: Minimum acceptable regimens for chemotherapy for participation in the CIBOMA study/2004-01

Type of Regimen	Regimens
ALL REGIMENS	To be eligible, the patient must have received **at least 6 cycles** of an approved (neo-) and/or adjuvant chemotherapy regimen. In all cases the **maximum** accumulative dose of doxorubicin (A) is 360mg/m^2 and of epirubicin (E) 720mg/m^2 **NOTE**: Patients without axillary node involvement are required to have received at least four cycles of adriamycin and cyclophosphamide as single chemotherapy treatment.
Anthracylines <u>without</u> taxanes	Among the permitted regimens in this category are included the combination of A or E with cyclophosphamide (C) with or without 5-fluorouracil (F), and A or E as single agents or combined with C followed by several cycles of CMF (where M is methotrexate). Initial minimum permitted dose of A or E associated with C and with or without F: CAF/FAC: 50mg/m2 (every 21 days or every 28 days) EC: 90mg/m2 75mg/m2 (administered day 1 every 21 days) if administered for a minimum of 6 cycles FEC/CEF: 50mg/m2 (administered days 1 and 8 every 28 days) $FE_{90}C$: 600/90/600 administered every 21 days or every 15 days. $FE_{100}C$: 500/100/500 administered every 21 days. 75mg/m2 (administered day 1 every 21 days) Permitted initial dose of A or E as single agents or combined with C followed by CMF (described below): A: 75mg/m2 E: 90mg/m2
Anthracyclines and taxanes 21-day regimens	Among the permitted regimens in this category are included the sequential or combined anthracyclines (A or E) and taxanes (paclitaxel [P] and docetaxel [D]). In sequential regimens, A or E can be combined with C and with or without F When anthracyclines (with or without C and/or F) and taxanes are administered sequentially, the initial minimum dose for injection of A is 60mg/m^2 (50mg/m^2 is also accepted for A in regimens of FAC); for E, 90mg/m^2 (75mg/m^2 is also accepted for E in regimens such as FEC); for P, 175mg/m^2/3-hours (75mg/m^2 every 7 days or 100 mg/m^2 every 7 days for 8 doses or 80 mg/m^2 every 7 days for 12 doses); and D, 100 mg/m^2 or 75 mg/m^2. When anthracyclines are administered (with or without C and/or F) together with taxanes, the initial minimum dose for injection of A is 50mg/m^2; for E, 75mg/m^2; for P, 135mg/m^2/3-hours; and for D, 60mg/m^2/1-hour or 75mg/m^2. Study scheme PACS 01: 3 cycles of $FE_{100}C$ followed by 3 cycles of docetaxel 100 mg/m^2 every 21 days.
Anthracyclines and taxanes 14-day regimens	The bi-weekly sequential regimen of anthracyclines and taxanes is accepted as follows: 4 cycles of doxorubicin 60mg/m^2 followed by 4 cycles of paclitaxel 175mg/m^2/3-hours, followed by 4 cycles of cyclophosphamide 600 mg/m^2/3.
GEICAM 9906	4 cycles of $FE_{90}C$: 600/90/600 administered every 21 days, followed by 8 weekly cycles of paclitaxel 100 mg/m^2.
Other regimens	Any regimen which forms part of a chemotherapy trial which has been previously approved by the Scientific Committee of CIBOMA.

APPENDIX 2: KARNOFSKY PERFORMANCE SCALE AND EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) CRITERIA

ECOG	KARNOFSKY	DEFINITIONS (Zubrod)
0	100	Absence of symptoms.
1	80-90	Symptomatic, completely occasional assistance.
2	60-70	Symptomatic, in bed less than 50% of the day.
3	40-50	Symptomatic, in bed more that 50% of the day, but not hospitalized.
4	20-30	Hospitalized.

APPENDIX 3: TNM CLASSIFICATION – BREAST (American Joint Committee on Cancer 2002)

PRIMARY TUMOR (T):

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis	(DCIS) ductal carcinoma in situ
Tis	(LCIS) lobular carcinoma in situ
Tis	Paget's disease of the nipple with no associated tumor. (Paget's disease associated with a tumor is classified according to the size of the tumor)
T1	Tumor 2.0 cm or less in greatest dimension
T1mic	Microinvasion 0.1 cm or less in greatest dimension
T1a	Not more than 0.5 cm in greatest dimension
T1b	Tumor more than 0.5 cm but less than 1.0 cm in diameter
T1c	Tumor more than 1.0 cm but less than 2.0 cm
T2	Tumor more than 2.0 cm but less than 5.0 cm in greatest dimension
T3	Tumor more than 5.0 cm in any dimension
T4	Tumor of any size with direct extension to chest wall or skin
T4a	Extension to chest wall (includes ribs, intercostal muscles, and serratus anterior muscle but not pectoral muscle)
T4b	Edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast
T4c	Both of the above (T4a and T4b)
T4d	Inflammatory carcinoma

LYMPH NODES (pN):

pNX	Regional lymph nodes cannot be assessed (previously removed or not removed)
pN0	No histological evidence of regional lymph node metastasis, no additional study for isolated tumor cells performed.
pN0(i-)	No histological evidence of regional lymph node metastasis, immunohistochemistry negative
pN0(I+)	No histological evidence of regional lymph node metastasis, immunohistochemistry positive with no cell aggregates >0.2 mm.
pN0(mol-)	No histological evidence of regional lymph node metastasis, no molecular findings on reverse transcriptase-polymerase chain reaction (RT-PCR)
pN0(mol+)	No histological evidence of regional lymph node metastasis, molecular findings on reverse transcriptase-polymerase chain reaction (RT-PCR) positive
pN1	Metastasis in 1-3 axillary lymph nodes and/or internal mammary chain with microscopic disease detected by sentinel node resection, but not clinically apparent*
pN1mi	Micrometastasis (greater than 0.2 mm and less than 2 mm)
pN1a	Metastasis in 1-3 axillary lymph nodes
pN1b	Metastasis in internal mammary chain with microscopic disease detected by sentinel node resection, but not clinically apparent*
pN1c	Metastasis in 1-3 axillary lymph nodes and in internal mammary chain with microscopic disease detected by sentinel node resection, but not clinically apparent*
pN2	Clinically apparent* metastasis in 4-9 axillary lymph nodes, or in internal mammary chain with no axillary node involvement.

pN2a	Metastasis in 4-9 axillary lymph nodes (at least one tumor deposit >2 mm)
pN2b	Clinically detectable* metastasis internal mammary nodes with no axillary node involvement.
pN3	Metastasis in 10 or more axillary nodes or in infraclavicular nodes; or in ipsilateral mammary chain, clinically apparent*, with involvement of 1 o more axillary nodes, or in more than 3 axillary nodes with microscopic metastatic involvement and not clinically apparent in the internal mammary chain; or in ipsilateral supraclavicular nodes.
pN3a	Metastasis in 10 or more axillary nodes or in infraclavicular nodes
pN3b	Clinically detectable metastasis in ipsilateral internal mammary nodes, with involvement of 1 or more axillary nodes, or in more than 3 axillary nodes and in internal mammary chain with microscopic disease detected on sentinel node resection but not clinically apparent.
pN3c	Metastasis in ipsilateral supraclavicular nodes.

***Clinically apparent, detectable: detected on imaging studies (except lymphoscintigraphy) or by clinical examination.**

DISTANT METASTASIS:

MX: Presence of distant metastasis cannot be assessed
M0: No evidence of distant metastasis
M1: Distant metastasis present

Breast cancer staging:

Stage 0	**Stage I**	**Stage IIA**	**Stage IIB**	**Stage IIIA**	**Stage IIIB**	**Stage IIIC**	**Stage IV**
TisN0M0	T1N0M0	T0N1M0	T2N1M0	T0N2M0	T4N0M0	Any TN3M0	Any T Any N M1
		T1N1M0	T3N0M0	T1N2M0	T4N1M0		
		T2N0M0		T2N2M0	T4N2M0		
				T3N1M0			
				T3N2M0			

APPENDIX 4: PATIENT INFORMED CONSENT MODEL

PRE-SCREENING INFORMED CONSENT: TRIPLE NEGATIVE TEST FOR CIBOMA STUDY/2004-01

INTRODUCTION AND OBJECTIVES

Your doctor is considering you for participation in a clinical study (also known as clinical trial) You will be able to take part in this trial once all the other treatments which you have been administered for your breast cancer have been completed. Before we continue talking about your possible participation in the study, other tests will first have to be carried out on your tumor sample, known as hormone receptor and HER2 expression tests. These tests can be carried out with a sample we already have, so it will not be necessary to carry out more biopsies, operations or blood draws.

The hormone receptor and HER2 expression tests will be carried out routinely in your hospital laboratory. If the result is negative, the tests will be repeated in a central laboratory in Madrid, Spain, to confirm the negative result.

In this study is promoted by the Iberoamerican Coalition for Research in Breast Oncology (CIBOMA - *Coalición Iberoamericano de Investigacíón en Oncología Mamaria*), non-profit organization which aim is to increase the quality and number of breast cancer clinical trials.

The aim of this document is to provide you with sufficient information for you to understand the possible risks and benefits which may be involved in these additional tests and confirmations. If you decide later to participate in the clinical trial, your doctor will ask you to read and sign a document which contains more details on the trial.

HORMONE RECEPTOR TEST

Breast tissue contains some hormone receptors, called estrogens and progesterones, which regulate many of the processes related with the feminine sex. Approximately 70% of breast cancers contain these receptors. These hormone receptors are absent from about 20% of tumors, and these tumors are called receptor negative.

In the case of your tumor, your doctor has determined using a technique called immunohistochemistry that it has negative hormone receptors, which is one of the two conditions necessary for your participation in the clinical trial.

HER2 TEST

HER2 is a protein which is produced in high levels in about 20% of breast cancer tumors. In approximately the other 80%, high levels of this protein are not detected. If you belong to this group (HER2 negative), you can participate in the clinical trial.

The negative result for hormone receptors and HER2 must be confirmed in the central laboratory. The laboratories will process the samples, using one of the following methods:

- Immunohistochemical staining (IHC)
- Fluorescence in situ hybridation (FISH).

ADDITIONAL TESTS

If you do participate in study CIBOMA 2004-01 in the future, another two tests will be carried out on the material sent to the laboratory, to determine if your tumor produces high levels of a protein called cytokeratin 5 and of another protein called HER1, which is also known as EGF receptor. The process which will be used for these tests is immunohistochemical staining.

POTENTIAL BENEFITS

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

It is possible that you do not obtain any personal benefit from the additional tests carried out on your tumor sample, although the information on the factors analyzed may be useful for directing your future treatment. Your doctor will provide you with the results of these tests.

ADDITIONAL COSTS OR PAYMENTS

You will not have to pay for the hormone receptor and HER2 tests, or for any additional test. Nor will you be paid for agreeing to the analyses.

SAMPLE HANDLING

Your sample will be identified by a numeric code during the study. When the study has finished, any reference to your identity will be eliminated (this numeric code will be eliminated) keeping the samples absolutely anonymous. From this moment and providing you do not require in writing the returning of the tumor sample from the pathological anatomy service of your hospital, it will be added to the CIBOMA breast tumor bank where it will be kept indefinitely. The aim of keeping these samples is to test future scientific findings. Some North American cooperative oncological groups have been operating this way between 10 and 20 years, and the tumor availability has let them test scientific hypothesis that have given relevant information for the breast cancer knowledge.

DATA CONFIDENTIALITY

You have a right to privacy and all the information which is collected for this study will be kept confidential within legal limits. In Spain, the handling of data of a personal nature is regulated by the Constitutional Law 15/1999, dated 13 December 1999. Your consent for handling your personal data and for allowing the use of the same can be revoked. You can exercise your right to access, rectification and cancellation, by asking your doctor, who will put you in contact with the sponsor. The personnel authorized by the sponsor for the study information managing, by your requirement, will be under the obligation to rectify or cancel those data of personal nature that you require.

If the hormone receptor and HER2 levels are positive, and you cannot participate in the clinical trial, your doctor will file your signed and dated pre-screening informed consent in your clinical records, along with the results of the tests and the medical observations from the pre-screening period.

If the hormone receptor and HER2 levels are negative, you will be able to participate in the CIBOMA 2004-01 clinical trial. In this case, your results will be collected by your doctor and sent to CIBOMA, Madrid, Spain, where the research database for the study is located.

Your original clinical records may be analyzed during or after the study by CIBOMA or its designated representatives, the health authorities and the local Ethics Committee representatives.

If you can, and you decide to, participate in the CIBOMA study, your data may be analyzed in any of the member countries of the Coalition, to determine how many patients have tumors with the same characteristics as yours (called triple-negative). CIBOMA may send the results to the health authorities of the CIBOMA countries which are participating in this study, present them in medical meetings, or publish them in specialized journals, so that other doctors may know about them. If you withdraw from the study, the information gathered about you up to the moment of withdrawal will continue to be used.

The representatives of CIBOMA, of the health authorities and of the local ethics committee may be permitted to inspect your clinical record to ensure that the information gathered is correct. In these cases, you may be identified personally.

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

Your participation in the study CIBOMA 2004-01 is described in another consent sheet.

OBTAINING ADDITIONAL INFORMATION

We encourage you to ask all the questions you want at any time during the study. If problems emerge or if you have more questions on prescreening, or on the study or your rights as a patient, call Dr…….. on ……..(tel.).

BASIS OF PARTICIPATION

The participation in prescreening procedures for the CIBOMA 2004-01 study is voluntary. If you do not want to participate, you will not lose any of the benefits to which you otherwise have a right nor will you suffer any penalty.

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

PATIENT DECLARATION AND SIGNATURE

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER

Study Number: CIBOMA/2004-01

I, _____

have read the information sheet which has been provided to me and I understand the aim of screening for the analysis of hormone receptor and HER2 expression.
I have been able to ask questions about the study.
I have received satisfactory answers to my questions.
I have received sufficient information about the study.

I have spoken with _____

I understand that my participation is voluntary.

I understand that I can withdraw from the study:

1. whenever I want,
2. without have to give explanations,
3. without affecting my medical care.

I authorize to the professional personnel of the sponsor of the clinical trial CIBOMA/2004-01, the Health Authorities or the pertinent International Bodies, to review my clinical and personal data related with the participation in this clinical trial.

I have been informed that I can revoke in any moment the authorization to review my clinical and personal data.
I freely give my agreement to participate in this screening for the analysis of hormone receptors and HER2 in a sample of my primary breast cancer.

_____

Date Signature of participant

I have fully explained the relevant details of this study to the above-named patient

_____

Date Signature of investigator

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER

Study Number: CIBOMA/2004-01

Your doctor has explained to you that you have breast cancer with a risk of recurrence. There are various treatments available which may help you. We are inviting you to participate in a research study with the medication Xeloda® (capecitabine, abbreviated to X), an oral medication which has been shown to be very effective in the treatment of advanced breast cancer. The trial is being carried out in various hospitals in Central/South America and in Spain. The participation of 876 patients is expected in this investigational study which has been approved by the authorities of your Hospital and the health authorities of your country. This study is sponsored by the Iberoamerican Coalition for Research in Breast Oncology (CIBOMA - *Coalición Iberoamericano de Investigacíón en Oncología Mamaria*), which is a non-profit-making association with the aim of increasing the quality and number of clinical trials in breast cancer.

OBJECTIVE

The objective of this study is to test if prolonging standard intravenous chemotherapy, which you have already received, with an oral chemotherapy treatment (capecitabine) is effective in preventing recurrence of your disease. You will have the same possibility of receiving treatment with 8 cycles of Xeloda® as not receiving any treatment and being assigned to an observation group. This will be decided by chance, like tossing a coin.

STUDY PROCEDURES

Before receiving treatment, you will have a series of tests to determine whether you can participate in the study. These tests will include blood tests and a physical examination. You may have to have a test of your heart function and radiological procedures to check that your disease has not spread to other parts of your body. We will also carry out routine tests on your tumor, for example, to check the status of hormone receptors and a protein called HER2. For these determinations to be consistent, you have previously allowed a sample of your tumor to be sent to a central laboratory, other than that of your own hospital. Your doctor may also request another blood sample from you in the case that you are assigned the capecitabine treatment arm to analyze the relation between your individual characteristics and a greater sensitivity to the study treatments. It is a pharmacokinetics study to determine the existence of polymorphisms in the thymidylate synthase enzyme (TS) and in the methylenetetrahydrofolate reductase enzyme (MTHFR), and to check their association with the toxicity and efficacy of the capecitabine treatment. To participate in this pharmacokinetic study, you may sign other informed consent form that your doctor will provide to you if you wish.

While you are on the study, and if you are in the capecitabine treatment group, you will have a blood test before the start of each new cycle (every 21 days). These regular blood tests and other tests will be carried out to check that the medications are not adversely affecting your bone marrow, kidneys and liver.

If you are assigned to treatment group A (capecitabine) you will have to receive treatment for 24 weeks (i.e. 8 cycles every 21 days). Every treatment cycle consists of 2 weeks of treatment followed by one week of rest. If you are assigned to group B (observation), you will not receive

treatment, but your doctor will ask you to come to see him/her routinely. Then, at the end of the study, your doctor will perform a follow-up examination.
After that, your doctor will follow you up in the same way as for other patients with breast cancer, to confirm that the tumor has not recurred.
For the first 2 years, you will have a physical examination every 3 months, and you will have a mammography and chest X-ray every 12 months.
During years 3 to 5, the three monthly visits will be dropped, but you will still have visits every 6 and 12 months, with the same evaluations.
The medication Xeloda® will be administered orally, twice a day (morning and evening) with water, 30 minutes after a meal. You should follow the treatment for 14 days in a row, after which it will be stopped for a week. Then you will start again, and so on, until the treatment is completed.

POSSIBLE ADVERSE EVENTS

The following side effects are the most common with the medication used in this study. These are side effects which we know about at the moment. However, since this is a study with new treatments, there may be other side effects that we still do not know about. For this reason, it is very important that you tell your doctor immediately about any unusual symptoms.
With Xeloda® you may experience diarrhea, abdominal pain, nausea, mouth irritation which might distress you when you are eating, and the so-called "hand-foot syndrome", which consists of numbness in your hands and feet, which may be accompanied by pain, flaky skin and discomfort which may affect your daily activities. Calcium levels in your blood may also be altered, but your doctor will check regularly for this. This drug is marketed, and we attach the product specifications, a document that states all the Xeloda® side effects.
These side effects may be small inconveniences or may be severe, but your doctor will monitor you closely and if anything happens, he/she will decide to adjust your chemotherapy dose or stop treatment. If treatment is discontinued, you will be offered an alternative treatment.
If you have fever or bruising after receiving any medication, you should contact the department doctors.

EXPECTED BENEFITS

You have already received treatment with standard intravenous chemotherapy before this study. You may also have received treatment with radiation therapy. With any of the standard treatments, a high percentage of women are completely cured of their breast cancer. This study will help us find out if prolonging treatment with chemotherapy increases this percentage of patients who are completely cured, or if in contrast, this does not happen, which is to say, that the breast cancer reappears, in the form of metastasis, in the same percentage of women who received standard treatment. If this happens, your doctor will ensure that you get the best treatment available for you at the time.
Your participation in this study may, then, not provide you with any benefit, but it will help us to understand the disease better, and we hope that we can use this information to help women who develop this disease in the future.

ALTERNATIVE TREATMENTS AVAILABLE

At the moment no alternative treatment is available in your situation and for your type of tumor. The usual procedure is that you do not receive more treatments, and that you are called for

routine check-ups, which is what will happen if you are assigned to the observation group of this study.

STUDY INSURANCE

In case you are injured during the study, CIBOMA has taken out an insurance policy with the HDI HANNOVER INTERNACIONAL (ESPAÑA) SEGUROS Y REASEGUROS S.A. Company with the number 130/101/000252 to cover third party responsibility of the study sponsor, the principal investigators and their co-workers, as well as the Hospital or Center Director, under the terms established in the Royal Decree 223/2004 which regulates the performance of clinical trials with medications in Spain. You will be informed of all significant new findings about Xeloda® which come to light during the study and which may make you change your mind about participating.

You must not take part in this study if you are pregnant or if there is a possibility that you may become pregnant during the study. Consequently, your doctor will check that you are using an effective contraceptive method before you start the study.

Your doctor may decide to withdraw you from the study if participation is not in your best interests, if you do not follow the instructions regarding the treatment, if it is discovered that you do not fulfill the trial requirements or if the study is terminated.

FINANCIAL ASPECTS

You should know that there is an economic compromise between the center and the principal investigator. However, it is not planned to pay you for your participation. It is considered the possibility to pay you the expenses of the extra visits to the hospital (those that will not be made under the habitual clinical practice), with a previous presentation of the supporting document.

VOLUNTARY PARTICIPATION

Your participation in this study is voluntary. You decide to participate but later change your mind, you are free to do so and you do not have to give a reason. However, you should tell your doctor about your decision, so that he/she can tell you what procedures should be followed to evaluate your clinical disease correctly and proceed with your medical care. The medical attention which you receive from your doctor will not be affected by your decision.

PEOPLE WITH ACCESS TO YOUR DATA AND CONFIDENTIALITY

If you participate in this study, your clinical records may be reviewed by professional staff from CIBOMA, from the Health Authorities or relevant international bodies. The data derived from your participation in the study may be published for scientific purposes, but your identity will be kept confidential, i.e. no-one will know your identity from the published data. The confidential nature of your personal data will be respected and guaranteed at all times, in accordance with the current regulations (Constitutional Law 15/1999, dated 13 December 1999 for the protection of data of a personal nature). You can exercise your right to access, rectification, cancellation and opposition by asking your doctor, who will put you in contact with the sponsor. The personnel authorized by the sponsor for the study information managing, by your requirement, will be under the obligation to rectify or cancel those data of personal nature that you require.

If you have any problem or question about the study, your rights as a clinical research participant or any research-related injury, you should contact:

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

Dr:.....tel:.....

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

WRITTEN PATIENT INFORMED CONSENT

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER

Study Number: CIBOMA/2004-01

I……

have read the information sheet provided to me
have been able to ask questions about the study
have received satisfactory answers to my questions
have received sufficient information about the study.

I have spoken with……..

I understand that my participation is voluntary.

I understand that I may withdraw from the study:

1. Whenever I want
2. Without having to give explanations
3. Without it affecting my medical care

I authorize to the professional personnel of the sponsor of the clinical trial CIBOMA/2004-01, the Health Authorities or the pertinent International Bodies, to review my clinical and personal data related with the participation in this clinical trial.

I have been informed that I can revoke in any moment the authorization to review my clinical and personal data.

I freely give my agreement to participate in this study.

Date Signature of Participant

Date Signature of Investigator

PATIENT AND INVESTIGATOR:
- SIGN AND DATE TWO COPIES OF INFORMED CONSENT
- KEEP ONE COPY IN CLINICAL RECORDS
- GIVE THE OTHER COPY TO THE PATIENT

APPENDIX 5.- INFORMATION SHEET AND INFORMED CONSENT TO PARTICIPATE IN THE PHARMACOGENETIC SUBSTUDY.

If you have decided to collaborate in the clinical trial **CIBOMA/2004-01** *"PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER"*, after having received the proper explanations about it, and having signed and dated the Informed Consent, your doctor will also give you the possibility to participate in a Pharmacogenetic Study of polymorphisms related with the efficacy and effectiveness of capecitabine. However, you may know that your decision to participate in this project does not affect to your participation in the referred clinical trial.

GENERAL INFORMATION ABOUT THE PHARMACOGENETICS UTILITY

Due to the fact that each person is different, cancer treatments do not have the same effect in the different patients. In some people, the therapy is shown to be more effective than in other, and the same happens with the side effects, some patients are more sensitive than others to them. The ideal situation would be that one that could identify the people that are candidate to receive treatment, because it would involve a profit that will exceed the risks of it, as well as to select the more effective drugs for each patient BEFORE the administration, by means of a tumor or blood analysis. To reach this situation, even many studies should be made with the aim to identify the genes and proteins involved in these drug response processes. For this reason, we request your collaboration in the pharmacogenetic study that we want to perform in parallel with the clinical trial that we are going to describe to you next.

DESCRIPTION OF THE SPECIFIC STUDY OBJECTIVES

If you decide to participate in this study, a blood sample will be extracted from you before the initiation of the treatment that will be analyzed for the study of an efficacy and side effects predictive factors to the treatment that you will receive during the clinical trial participation.

Particularly, what it is intended is to isolate DNA from white blood cells and blood plasma. These DNA samples will be studied to determine the existence of a series of polymorphisms (genes that vary their sequence in different individuals) related to the capecitabine efficacy and toxicity.

Results of previous studies let to propose the hypothesis that the existence of particular polymorphisms of the gene that codify for the thymidylate synthase enzyme (TS) and the gene that codify for the methylenetetrahydrofolate reductase enzyme (MTHFR), can predict which patients are going to experience toxicities and will require capecitabine dose reductions and which patients will not take profit from the standard capecitabine dose.
The aim of this substudy is to prove this association.

POTENTIAL, INDIVIDUAL AND/OR COLLECTIVE BENEFITS

As in the majority of cases, can be no potential benefits for you in your participation in this substudy. However, greater knowledge about the factors involved in the development of breast cancer or in its resistance to known drugs, could help the administration in the future of an individual treatment for each patient, that minimize the impact of the side effects by increasing its success probabilities.

This research does not intend to give you or your doctor new information about your genetic status. The results of these tests will not be used to evaluate your disease. This is a research study and will not give reliable clinical information that would help your doctor to make informed decisions about your health or to determine a suitable treatment for you.

POSSIBLE PERSONAL AND/OR FAMILIAR RISKS DERIVED FROM THE PARTICIPATION

The possible medical risks derived from the participation in this substudy are the ones associated to the extraction of one blood sample and that consist on the possible local complications (bruises, small scabs), that could raise after the procedure.

VOLUNTARY PARTICIPATION IN THE STUDY AND POSSIBILITY TO WITHDRAW

Your participation in this study is voluntary. If you decide to participate but later change your mind, you are free to do so, and you do not have to give a reason. However, you should tell your doctor about your decision, and he/she will take care of the destruction or the total anonymization of your sample (not vinculated with the assigned number).

STUDY PROCEDURES

If you decide to participate in this substudy, you will only have to give your consent. You do not need to do anymore.

SAMPLE HANDLING

From the extraction moment, your sample will be identified only with a number during all the study development.

PATIENT RIGHT TO THE ACCESS AND RECTIFICATION OF THE GENETIC INFORMATION

You can exercise your right to access, rectification, cancellation and opposition by asking your doctor, who will put you in contact with the sponsor. The personnel authorized by the sponsor for the study information managing, by your requirement, will be under the obligation to rectify or cancel those data of personal nature that you require.

The obtained information will be kept confidential and codified until the study close-out, after this it will become anonymous (the assigned number will be eliminated).

EXPENSE PAYMENTS TO THE PATIENTS

Due to that it is not considered that the substudy participation may produce additional travels to the hospital, the costs for these expenses will not be paid.

COMMERCIAL AND PATENT INTERESTS DERIVED FROM THE STUDY

This study does not involve any commercial interests, all the interests are completely scientific. However, we may remind you that if this study would give place to an industrial or commercial patent you will not take any profit from it.

CONFIDENTIALITY

The information collected as part of this study will be shared with other investigators and doctors. However, a strict confidentiality will be kept, and you will not be identified by your name in any of the collected data and materials during the study. The Spanish health authority agents, the member of the of your hospital's Clinical Research Ethics Committee, or the monitors or agents of the sponsor (CIBOMA) could need to consult your clinical record in relation to your study participation. This is part of the quality control process. Every person that consult your record will follow the relevant rules in each center and the proper procedures.

In case that you have any problem or any question about the study, your rights as a participant in a clinical trial or about any injure related to the research, you may contact:

Dr..... Tel:.....

PATIENT CONSENT IN WRITING

PHARMACOGENETIC SUBSTUDY OF THE CLINICAL TRIAL:

PHASE IV.III, MULTICENTER, OPEN, RANDOMIZED TREATMENT STUDY TO EVALUATE THE EFFICACY OF MAINTENANCE THERAPY WITH CAPECITABINE (X) AFTER STANDARD (NEO-) AND/OR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH OPERATED, HORMONE RECEPTOR AND HER2neu NEGATIVE BREAST CANCER

Study number: CIBOMA/2004-01

I, ...

Have read the information sheet provided to me
Have been able to ask questions about the study
Have received satisfactory answers to my questions
Have received sufficient information about the study.

I have spoken with .....

I understand that my participation is voluntary.

I understand that I may withdraw from the study:

1. Whenever I want
2. Without having to give explanations
3. Without affecting my medical care

I authorize to the professional personnel of the sponsor of the clinical trial CIBOMA/2004-01, the Health Authorities or the pertinent International Bodies, to review my clinical and personal data related with the participation in this substudy.

I have been informed that I can revoke in any moment the authorization to review my clinical and personal data.

I freely give my agreement to participate in this study.

Date Signature of participant:

Date: Signature of investigator:

APPENDIX 6: ADVERSE EVENT REPORT FORM

REPORT OF SUSPECTED ADVERSE REACTION FOR **INVESTIGATIONAL MEDICATIONS**	PROTOCOL CODE (SPONSOR)	NOTIFICATION NUMBER (SPONSOR)
	PATIENT NUMBER	NOTIFICATION NO.

I. ADVERSE REACTION INFORMATION

1a. COUNTRY	2. DATE OF BIRTH			2a. AGE	3. SEX	3a. WEIGHT	3b. HEIGHT	4-6. DATE OF ONSET OF REACTION		
	DAY	MONTH	YEAR		☐ MALE ☐ FEMALE			DAY	MONTH	YEAR
7. DESCRIPTION OF ADVERSE REACTION (Including relevant results of physical or laboratory tests, date of resolution, if applicable).					8-13b. CRITERIA FOR SERIOUSNESS/OUTCOME ☐ DEATH ☐ LIFE-THREATENING ☐ HOSPITALIZATION ☐ PROLONGATION OF HOSPITALIZATION ☐ PERMANENT OR SIGNIFICANT DISABILITY ☐ CLINICALLY SIGNIFICANT AR ☐PERSISTENCE OF ADVERSE REACTION ☐ RESOLUTION					

II. STUDY MEDICATION DATA

14.SUSPECTED MEDICATION	15. DAILY DOSE	16. ROUTE	17. DISEASE UNDER STUDY	18. DATES OF ONSET END		19. DURATION OF TREATMENT

20. DID THE REACTION SUBSIDE WHEN MEDICATION WAS SUSPENDED?	20a. DID THE REACTION SUBSIDE WHEN DOSE WAS REDUCED	21. DID THE REACTION REAPPEAR ON RECHALLENGE?
☐ YES ☐ NO ☐ NOT APPLICABLE	☐ YES ☐ NO ☐ NOT APPLICABLE	☐ YES ☐ NO ☐ NOT APPLICABLE

III. CONCOMITANT MEDICATIONS AND CLINICAL HISTORY

22.CONCOMITANT MEDICATIONS (Indicate suspected medication(s) with an asterisk)	22a. DAILY DOSE	22b. ROUTE	22c. DATES OF ONSET END	22d. REASON FOR PRESCRIPTION

23. CLINICALLY SIGNIFICANT DATA FROM CLINICAL HISTORY (e.g. diagnoses, allergies, pregnancies, etc)					

IV. SPONSOR AND INVESTIGATOR DATA

24a. NAME AND ADDRESS OF SPONSOR		24b. NAME AND ADDRESS OF INVESTIGATOR
24c. LABORATORY CODE (Spanish Drug Agency No.)	25a. TYPE OF REPORT YINITIAL YFOLLOW-UP	24c. SPONSOR CONTACT MAKING REPORT NAME: TELEPHONE: SIGNATURE:
24e. DATE OF REPORT	24f. DATE OF RECEIPT IN SPANISH DRUG AGENCY	25b. COMPLEMENTARY REPORT ATTACHED

APPENDIX 7: LIST OF PARTICIPANT COUNTRIES

In Spain: The following investigators have shown their interest in participating in this study. Their incorporation to the study will be made at intervals.
Centers from Argentina, Brazil, Chile, , Guatemala, Mexico, Peru, Uruguay, Venezuela, Ecuador and Colombia are also expected to participate in the study.

INVESTIGATOR	HOSPITAL
Jesús García Mata	H. Sta. María Nai
José Ramón Mel Lorenzo	H. Xeral Calde
Laura de Paz	H. Arquitecto Marcide
Javier Castellanos	H. Xeral Cíes
Manuel Constela	H. Montecelo
Manuel Ramos	Centro Oncológico Galicia
Lourdes Calvo Martínez	Complejo Hospitalario Universitario A Coruña
Concepción Almanza Madera	Policlínico de Vigo POVISA
Dolores Menendez Prieto	C.Hospit.Univ. Santiago
Santiago Albiol Rodríguez	H. Del Espiritu Santo
Agustín Barnadas i Molins	H. de la Santa Creu i Sant Pau
Sonia González	H. Mutua Terrassa
Rosa Mª Franquesa Grané	H. General de VIC
Miguel Angel Segui	Hospital Parc Taulí
Montserrat Muñoz Mateu	H. Clínico y Provincial
Alfonso Modolell	Ins. Oncológico Corachán
Amadeu Pelegrí	Hospital Sant Joan de Reus
Miguel Gil	Instituto Catalán de Oncología
Angels Arcusa Lanza	Consorci Sanitari de Terrassa
Ignacio Tusquets Trias de Bes	Hospital del Mar
Mireia Margelí	H. Germans Trias i Puyol
Ramón Colomer Bosch	H. Josep Trueta
Isabel Moreno Solórzano	H. Municipal de Badalona
Ana de Miguel	H. San Juan de Dios de Manresa
Antonio Llombart	H. Arnau de Vilanova
Montserrat LLobera	H. Tortosa Verge de la Cinta
Montserrat Boleda	H. Sant Camil
Antonio Fernández Aranburo	Hospital General de Albacete
Javier Cassinello	H. Gral. de Guadalajara
Miguel Ángel de la Cruz Mora	H. Virgen de la Salud
Pedro Puñal	H. Provincial de Toledo
Mª del Mar Muñoz	H. Virgen de la Luz
Encarna Adrover	H.Gral. Univ. de Alicante
Mª José Godes Sanz Bremond	H. General de Valencia
Vicente Carañana	H. Arnau de Vilanova
Amparo Ruiz Simón	I.V.O.
Alvaro Rodriguez Lescure	H. General de Elche
José Miguel Cuevas	Hospital de la Ribera
Ana Lluch	H. Clínico de Valencia
Antonio Galán Brotons	H. Puerto de Sagunto
Miguel Pastor Borgoñón	H. Universitario la Fe

Santiago Olmos	H. Universitario Dr. Peset
Cristina Llorcas	H. General de Elda
Adolfo Frau Llopis	H. Provincial Castellón
José Lizón Giner	H. Clínico San Juan
Mª Dolores Torregrosa	H. Lluis Alcanys
Amparo Oltra	H. Virgen de los Lirios
Norberto Batista	H. Universitario de Canarias
Adolfo Murias Rosales	Hospital Insular
José Aguiar Morales	H. Gran Canaria Dr. Negrin
Maria del Pilar López Álvarez	C. Hosp. Ntra. Sra. De la Candelaria
José Ignacio Mayordomo	H. Clinico Univ. de Zaragoza
Jesús Florián Jericó	H. Comarcal Barbastro
Antonio Antón Torres	H. Miguel Servet
Alfonso Yubero	H. Gral de Teruel O. Polanco
José Ángel García Sáenz	H. Clínico San Carlos
Ricardo Cubedo	Clínica Puerta de Hierro
Miguel Ángel Lara Álvarez	H. Severo Ochoa
Miguel Méndez Ureña	H. General de Móstoles
José Enrique Alés Martínez	H. Ruber Internacional
Carlos Jara Sánchez	Fundación Hosp. Alcorcón
Eduardo García Rico	Hospital de Madrid
Eduardo García Rico	Hospital de Montepríncipe
Gumersindo Pérez Manga	H. Gregorio Marañón
César Mendiola Fernández	H. Doce de Octubre
Carmen Crespo	H. Ramón y Cajal
Francisco Lobo Samper	Fundación Jimenez Díaz
Santos Enrech Francés	Hospital Gomez Ulla
Amalia Velasco Ortiz de Taranco	Hospital la Princesa
Ramón Pérez Carrión	Clínica MD. Anderson
Laura G. Estévez	Hospital Madrid Norte Sanchinarro (CIOCC)
Javier Salvador Bofill	H. U. De Valme
Manuel Ruiz Borrego	H. Univ. Virgen de Rocio
José Mª Baena Cañada	H. Puerta del Mar
Juan Rafael de la Haba	H. Reina Sofía
María Lomas Garrido	Complejo Hospitalario de Jaén
Liliana Canosa	Hospital Torrecardenas
Antonio Lorenzo Peñuelas	H. Univ. Puerto Real
Encarnación González Flores	H. Virgen de la Nieves
Alberto Luis Moreno Vega	H.Gral de Jerez Frontera
Juan Lucas Bayo Calero	H. Juan Ramón Jimenez
Pedro Valero Jimenez	Clínica Sagrado Corazón
Rafael Trujillo	Hospital Punta de Europa
José Luis García Puche	H. Clínico San Cecilio
Emilio Alba Conejo	H. U. Virgen de la Victoria
Ester Villar Chamorro	C.H. Carlos Haya
Arrate Plazaola Alcibar	Onkologikoa
Isabel Alvarez López	Hospital Donostia
J. Ramón Barceló	Hospital de Cruces
Purificación Martinez Prado	Hospital de Basurto
Severina Dominguez	Hospital Txagorritxu

José Luis Alonso Romero	H. Virgen de la Arrixaca
Francisco Ayala de la Peña	H. Morales Meseguer
José Manuel López Vega	Hops. U. Marques Valdecilla
López Lara	H. Clínico Univ. Valladolid
Alberto Arizcum	Hospital Rio Carrión
César Rodriguez Sánchez	H. Universitario Salamanca
García Palomo	H. General de León
Blanca Hernando	H. General Yague
José Valero Alvarez Gallego	H. Rodriguez Chamorro
Juan Carlos Torrego	H. Univ. del Rio Hortega
Florestán Juarez	H. Ntra. Senora del Prado
Mª Lomas Garrido	Hospital Infanta Cristina
Pablo Borrega	H. San Pedro Alcantara
Yolanda López del Puerto	H. Virgen del Puerto
Mª Ángeles Panadero	H. Ciudad de Coria
Antón Avellá	H.Son Dureta
José Juan Irramendi Mañas	Hospital de Navarra
Edelmira Velez de Mendizabal María Valero Arbizu	Hospital Infanta Luisa de Sevilla

APPENDIX 8: RECORD OF MENSTRUAL STATUS

RECORD TABLE OF MENSTRUAL STATUS

INSTRUCTIONS: Use **B** if there is bleeding but without need for sanitary protection.
Use **T** if you need sanitary protection (towels, tampons, panty-liners, etc).
Leave blank if there is no bleeding.

Date of starting record (M, D, Y)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Jan																															
Feb																															
Mar																															
Apr																															
May																															
Jun																															
Jul																															
Aug																															
Sept																															
Oct																															
Nov																															
Dec																															

COMMENTS:_____
_____
_____

_____
Please write your name in block capitals

INVESTIGATOR: Keep a copy for your record

APPENDIX 9: COCKROFT AND GAULT CRITERIA

Cockroft-Gault formula for women:

Creatinine clearance (ml/min) =
[(140 – age) x current body weight (in Kg) x 0.85] / [72 x serum creatinine (in mg/dL)]
o
[(140 – age) x current body weight (in Kg) x 0.85] / [0.81 x serum creatinine (in µmol/L)]

APPENDIX 10: CAPECITABINE DOSE CALCULATION IN RELATION TO THE BODY SURFACE AREA (ONLY TABLETS OF 500 MG).

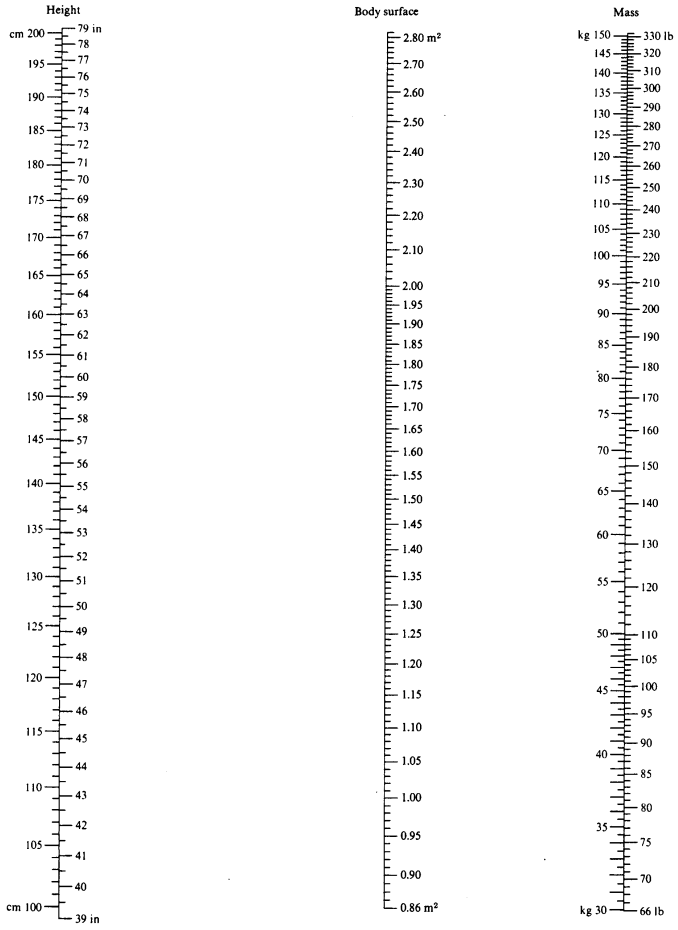
The initial dose of 100% corresponds to the administration of 1,000 mg/m^2 twice a day (total daily dose of 2,000 mg/m^2).

Dose level of 100% = 1000 mg/m^2 twice a day		Number of tablets of 500 mg	
Body surface area (m^2)	Average dose per administration (mg)	Morning	Evening
≤ 1.12	1,000	2	2
1.13 – 1.37	1,250	2	3
1.38 – 1.62	1,500	3	3
1.63 – 1.87	1,750	3	4
≥ 1.88	2,000	4	4

Dose level of 75%		Number of tablets of 500 mg	
Body surface area (m^2)	Average dose per administration (mg)	Morning	Evening
≤ 1.12	750	1	2
1.13 – 1.37	1,000	2	2
1.38 – 1.87	1,250	2	3
≥ 1.88	1,500	3	3

Dose level of 50%		Number of tablets of 500 mg	
Body surface area (m^2)	Average dose per administration (mg)	Morning	Evening
≤ 1.12	500	1	1
1.13 – 1.87	750	1	2
≥ 1.88	1,000	2	2

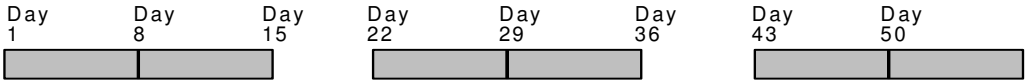
Nomogram for determination of body surface from height and mass[1]



[1] From the formula of Du Bois and Du Bois, *Arch. intern. Med.*, 17, 863 (1916): $S = M^{0.425} \times H^{0.725} \times 71.84$, or $\log S = \log M \times 0.425 + \log H \times 0.725 + 1.8564$ (S: body surface in cm², M: mass in kg, H: height in cm).

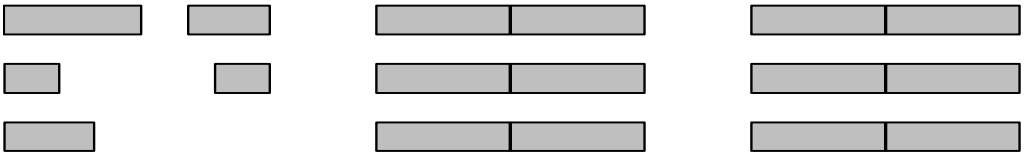
APPENDIX 11: DISCONTINUANCE AND EXTENSION OF THE REST PERIOD IN THE CAPECITABINE TREATMENT

Normal treatment:



Discontinuances during treatment:

The discontinuances are considered as lost doses. The initial treatment plan will be kept.



Rest period extension:

If a rest period is extended due to toxicity, the complete cycle will be administered later.