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"Docetaxel in adjuvant therapy of breast cancer: results of the *TAXIT 216* multicenter phase III trial"

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“Docetaxel in adjuvant therapy of breast cancer: results of the *TAXIT 216* multicenter phase III trial”

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LIST OF PUBLICATIONS

This dissertation is based upon the following publications:

The Taxit 216 Investigators (including **Forestieri V.**). Sequential Epirubicin-Docetaxel-CMF as adjuvant therapy of node-positive early stage breast cancer: the Taxit 216 randomized trial. In preparation.

De Laurentiis M, Cancello G, D'Agostino D, Giuliano M, Giordano A, Montagna E, Lauria R, **Forestieri V**, Esposito A, Silvestro L, Pennacchio R, Criscitiello C, Montanino A, Limite G, Bianco AR, De Placido S. Taxane-based combinations as adjuvant chemotherapy of early breast cancer: a meta-analysis of randomized trials. Journal of Clinical Oncology 2008; 26:44-53.

The references to the above papers are referred in the body text. The articles are attached at the end of the dissertation.

ABSTRACT

The dissertation is focused on the evaluation of a Docetaxel-based sequential regimen as adjuvant therapy of breast cancer.

Docetaxel is among the most active chemotherapeutic agents for breast cancer. With Taxit 216 trial, we aimed to assess the efficacy of adding docetaxel in a block-sequential fashion to a regimen with doxorubicin followed by CMF in the adjuvant therapy for node-positive early stage breast cancer (ESBC).

Patients were randomized to arm A (epirubicin 120 mg/m^2 for 4 cycles then cyclophosphamide, methotrexate and 5-fluorouracil [CMF] for 4 cycles), or arm B (4 cycles of docetaxel 100 mg/m^2 administered after the fourth epirubicin cycle and before the first CMF cycle). Treatment allocation was performed, in our Institution, by a computer program using a minimization algorithm. Stratification factors were: center, lymph node involvement (1 to 3, 4 to 9, >10), estrogen receptor status (negative/positive/unknown), menopausal status (pre/post). The primary end-point was invasive disease-free survival (IDFS). Secondary end-points were recurrence-free survival (RFS), overall survival (OS) and toxicity. The study was designed to detect a hazard ratio of 0.70, assuming an α of 0.05 (two-sided), a power of 0.80 and an expected DFS in arm A of 0.65 at 5 years. This required 480 patients per arm and 250 events. Final results are reported according to the standardized system for efficacy end points (STEEP system).

Between July 1998 and July 2002, 972 patients were randomized (486 in each arm). At a median follow-up of 62 months, 278 IDFS events were recorded. Five-year IDFS was 74% in arm B *vs* 68% in arm A ($P = 0.13$), with a hazard ratio (HR) of 0.82 (95% confidence interval [CI] = 0.64–1.03). RFS was significantly better for arm B than for arm A (76% *vs* 69%; $P = 0.0332$) with a HR of 0.75 (95% CI = 0.59–0.96). There was a significant improvement in OS, with an estimated five-year OS of 90% in arm B and 85% in arm A ($P = 0.0168$; HR = 0.67, 95% CI = 0.48–0.94). This benefit comes at the cost of increased but acceptable toxicity.

We demonstrated, with the results of the Taxit 216 phase III trial, that incorporating docetaxel into a block-sequential epirubicin–CMF regimen significantly reduces the risk of recurrence and death in patients with node-positive ESBC.

1 INTRODUCTION

1.1 Adjuvant chemotherapy

An overview, carried out in 1998 by the Early Breast Cancer Trialists' Collaborative Group, of all randomized trials performed worldwide demonstrated that adjuvant chemotherapy reduces risk of recurrence and death of operable early stage breast cancer (ESBC) (EBCTCG 1998). The overview also showed that anthracycline-based combinations are generally more effective in ESBC than earlier combinations, like cyclophosphamide-methotrexate-fluorouracil (CMF); in fact, there was an additional reduction in the annual breast cancer relapse rate of 12% and of the annual death rate of 11% versus CMF. These findings encouraged the diffusion of anthracycline-based regimens but, in the absence of direct comparison between different anthracyclines-based schedules, different regimens were adopted worldwide, based on local preference.

In this scenario, a sequential regimen consisting of 4 courses of doxorubicin followed by various courses of CMF gained widespread acceptance in Europe. This was fuelled by the results of a randomized trial by Bonadonna et al (Bonadonna et al 1995, Buzzoni et al 1991), in which this block-sequential regimen compared favorably with a regimen alternating doxorubicin and CMF courses. This observation was also consistent with mathematical models that predicted better outcomes with block-sequential therapy than with an alternating regimen of non-cross-resistant agents (Norton and Simon 1986). Therefore, despite the lack of trials directly comparing the block-sequential therapy with the classical CMF, this regimen was regarded as a standard treatment by many clinicians in Europe and was, thus, chosen as standard reference arm by the Taxit 216, designed by Italian investigators in 1998 in the attempt to improve the efficacy of a standard adjuvant chemotherapy for node-positive early stage breast cancer. Nonetheless, the superiority of the block-sequential regimen over classical CMF has been more recently demonstrated for both anthracycline compounds, doxorubicin (De Placido et al 2005) and epirubicin (Poole at al 2006), thus providing further support to the reference arm in our trial.

In the Taxit 216 we aimed to assess the efficacy of adding docetaxel to a sequential anthracycline-based regimen in the adjuvant therapy for node-positive early stage breast cancer (ESBC). In accordance with the Norton-Simon model (Norton and Simon 1986), this regimen should warrant the highest dose-intensity for each drug used at standard dose while theoretically limiting the increase of toxicity, thus possibly yielding the best chance of tumor eradication.

1.2 Taxanes

In the late 1960's the National Cancer Institute's large-scale plant screening program found that a crude extract of the bark from the Pacific yew, *Taxus brevifolia* had activity against the P388 mouse leukemia. In 1971, Wani, Taylor et al (Wani et al 1971) isolated and characterized Taxol (paclitaxel), the active principle of the extract. Subsequent research showed that paclitaxel has activity against several human malignancies including refractory ovarian cancer and breast cancer (Holmes et al 1971) and Taxol (Bristol Myers Squibb) is now approved for use in these indications in some countries.

Several years ago, researchers at Rhône-Poulenc Rorer, with the co-operation of the French "Center National de Recherche Scientifique (CNRS)", were able to prepare docetaxel, a semisynthetic analog of paclitaxel, using a precursor extracted from the needles of the European yew, *Taxus baccata*. Docetaxel was shown to have superior *in vivo* antitumor activity as compared to paclitaxel in the B16 melanoma model (Bissery et al 1990). Docetaxel also has a slightly better solubility than paclitaxel. Its toxicity profile in animals was favorable, and it has subsequently been undergoing development in an international human clinical trial program. Development has reached the stage of phase II/III clinical trials and has shown favorable response rate in breast cancer.

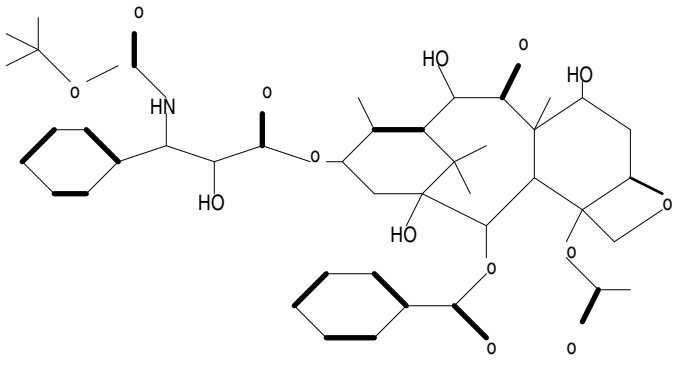
1.3 Docetaxel

1.3.1 Name and chemical information

- Docetaxel, (RP 56976)

- Chemical name: 4-acetoxy-2a-benzoyloxy-5b, 20 -epoxy-1, 7b, 10b-Trihydroxy- 9-oxotax-11 -ene- 13a-yl -(2R, 3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate

- Chemical structure:



- Empirical formula: $C_{43}H_{53}O_{14}N$, 3 H_2 O
- Molecular weight: 807.9
- Appearance: White powder
- Solubility at 20°C: water practically insoluble

1.3.2 Mechanism of action

Docetaxel has a mechanism of action which is similar to (or may be identical to) paclitaxel (Bissery et al 1990). Docetaxel enhances microtubule assembly and inhibits the depolymerization of tubulin. As with paclitaxel, this can lead to bundles of microtubules in the cell, which by blocking cells in the M phase of the cell cycle results in the inability of the cells to divide. This contrasts with the action of other spindle poisons in clinical use such as colchicine or vinca-alkaloids which inhibit tubulin assembly in microtubules.

Comparing paclitaxel and docetaxel using the "tubulin *in vitro* assay", the concentration required to provide 50% inhibition of microtubule disassembly (or IC50) for docetaxel is 0.2 µm and for paclitaxel is 0.4 µm.

1.3.3 Experimental antitumor activity

Docetaxel has been tested against tumors representing a variety of tissue types and behavioral patterns. It is highly active against B16 melanoma. The total log cell kill is 2.5 times greater for docetaxel than for paclitaxel, at equitoxic dosages in this model. Docetaxel is active against three colon tumors: C38, C51 and C26, and causes complete regression of advanced stage colon adenocarcinoma C38. It also causes complete regression of advanced stage pancreatic adenocarcinoma P03. Docetaxel exhibits cross resistance to pleiotropic resistant cell lines. Docetaxel is considered as a schedule independent drug: anti-tumor activity correlates with the total dosage that can be administered and dose-splitting does not appreciably change efficacy.

1.3.4 Human pharmacokinetic data

The docetaxel kinetic profile is consistent with a three-compartment pharmacokinetic model independent of administration schedule or dose, although the terminal elimination phase can not always be observed i.e. at low doses and/or for some administration schedules because of the low plasma levels achieved. No evidence of dose-dependence of docetaxel clearance (CL) was observed following either 1-2 hour (TAX 001) or 6 hours (TAX 004) infusions. Typical drug exposure following 1 hour infusion of 100 mg/m² is (TAX 006 study) :
- Peak: 3.67 µg/ml

- AUC: 4.59 µg.h/ml

Mean pharmacokinetic parameter estimates are (TAX 001 and TAX 006 studies, population analysis):

- t1/2 : 4 min
- t1/2 : 36 min
- t1/2 : 11.1 h
- CL : 35.3 l/h (21.0 l/h/m²)
- Vss : 113 l (67.3 l/m²)

Docetaxel is extensively bound to plasma proteins. In vitro the plasma protein binding is 93-94 % whatever the drug concentration. The main proteins involved are albumin, 1-acid glycoprotein and lipoproteins. A binding of 97.8% was measured in vivo in 3 cancer patients. None of the anticancer drugs studied nor dexamethasone were found to displace docetaxel binding.

After infusion of 14C-docetaxel (100 mg/m² in 1 hour), excretion occurs mainly in the feces (75% of the dose) mostly during the first 48 hours post dosing. Urinary excretion accounts for only 5% of the dose. Docetaxel is extensively metabolized and unchanged drug represents a small fraction of the radioactivity excreted. However, most of the circulating radioactivity is accounted for by unchanged docetaxel, and no circulating metabolites could be detected in plasma. The main metabolic pathway for docetaxel metabolism in humans as in animal species consists of successive oxidations (alcohol, aldehyde, acid) of the tert-butyl ester group on the side chain.

A sparse sampling strategy aiming at defining the docetaxel kinetic profile over a population of patients and assessing docetaxel population PK/PD was implemented in 22 Phase II multi-centric studies (577 patients). Population PK analysis demonstrated that inter-patient variability of docetaxel clearance is significantly related to body surface area, age, 1-acid glycoprotein and albumin plasma levels and hepatic enzyme plasma levels. The presence of liver metastases per se was not found to alter clearance. Likewise, clearance was not found to differ between male and female patients. PK/PD analysis (logistic regression model) using estimates of individual patient CL demonstrated that, after adjustment for the effects of other covariates, CL variability is a strong predictor of the odds of Grade 4 neutropenia.

1.3.5 Efficacy in advanced breast cancer

Taxanes were introduced for treatment of advanced breast cancer in the 1990s and their potential utility as adjuvant therapy was well acknowledged in 1998. Docetaxel, in particular appeared to be highly effective compound as monochemotherapy in the treatment of metastatic breast cancer and its activity in anthracycline-resistant disease was well defined.

Phase II studies showed a response rate raging from 48.2% in anthracycline resistant patients to 38.8% in anthracycline refractory patients (Valero et al 1995; Ravdin et al 1995). These data were confirmed by the phase III studies conducted in anthracycline failure patients (Chan et al 1999, Paridaens et al 2000, Sledge et al 2003). Infact, two international phase III studies were conducted to compare the antitumor activity of taxotere administered as monotherapy, to doxorubicin and to a combination of mitomycin C and vinblastine respectively (study 303, 304) (Nabholtz et al 1999). Three hundred and twenty-six patients with metastatic breast cancer who failed previous alkylating agents entered in study 303; 161 patients were randomized to receive 100 mg/m^2 taxotere every 3 weeks and 165 patients received 75 mg/m^2 of doxorubicin every 3 weeks. Both drugs were administered for a maximum of 7 cycles. The main patient characteristics (median age, performance status, previous hormonal treatments and previous response to alkylating agents, site of disease, and number of sites involved) were well balanced in the two treatment groups. In the intention to treat population the overall response rate (ORR) is significantly higher ($P \leq 0.004$) in taxotere group (47%) with respect doxorubicin group (32%) with 8.1% CR in taxotere group *vs* 4.2% in doxorubicin group. An earlier onset of the response was observed in taxotere treated patients 13 wks (range for the responders 3 - 51) *vs* 23 wks (range for the responders 3 - 23) in doxorubicin patients. Higher response rate was observed in patients resistant to alkylating agents, 43% in taxotere group *vs* 21% in doxorubicin ($P \leq 0.003$) and with visceral metastases (46% in taxotere group *vs* 28% in doxorubicin group $P \leq 0.003$). The difference between the 2 treatment groups was particularly striking in the subgroup of patients with liver metastases; a response rate of 57% was achieved in taxotere treated patients whereas only the 23% of patients responded in doxorubicin group ($P < 0.001$). To date, no sufficient events are available to conduct the analysis on TTP and survival. The median number of cycles administered were 7 in taxotere group and 6 in doxorubicin group with a median relative dose intensity (RDI) of 0.97 (0.05-1.07) for taxotere and 0.95 (0.49-1.05) for doxorubicin. Three hundred and twenty-two patients out of 326, 159 in taxotere arm and 163 in doxorubicin arm were evaluable for safety. Two patients in each group were randomized but did not receive the treatment. The 12 % and the 14% of the patients discontinued the study drug for adverse events in taxotere and doxorubicin group respectively. The study drug assigned was discontinued for cardiac toxicity in all patients in doxorubicin group, whereas in taxotere group neurological toxicity and fluid retention leaded to drug discontinuation in 7 and 3 patients respectively. Treatment discontinuation for death occurred in 5 patients (3%) in taxotere and in 4 patients (2%) in doxorubicin group. One septic death occurred in both groups; 2 and 1 patients died of unrelated events in taxotere and doxorubicin group respectively. All the other deaths were due to progressive disease. The most frequent adverse event reported in both arms was neutropenia. No significant differences were reported about the incidence of this side effect (97.4% and 96.7%) including grade 3 and 4 episodes (grade

3 - 14.4% vs 10.6%; grade 4 - 78.4% vs 78.1%) in taxotere and doxorubicin treated patients. The percentage of febrile neutropenia was significantly higher P≤ 0.04 in doxorubicin arm 12.3% with respect taxotere 5.7% as well as the incidence of thrombocytopenia 39.4% vs 3.8%, although the incidence of grade 4 thrombocytopenia was low in both groups (1.3% vs 7.5% in taxotere and doxorubicin arm respectively). The main grade 3 and 4 non hematological side effects experienced by patients in taxotere group versus doxorubicin group were: nausea 2.5% vs 14.1%; vomiting 2.5% vs 11.7%; diarrhea 10.7% vs 1.2%; stomatitis 5% vs 11.7%; asthenia 13.8% vs 11.7%. Severe fluid retention and neurosensory disorder occurred in 5% of patients treated with taxotere no death related to these adverse events were reported; 3.1% of patients in doxorubicin group experienced cardiotoxicity which was responsible for 2 deaths. Three hundred and ninety-two patients with metastatic breast cancer who failed previous anthracycline agents entered in study 304. Two hundred and two patients were randomized to receive 100 mg/m^2 taxotere every 3 weeks and 190 patients received a combination of Mitomycic C 12 mg/m^2 every 6 weeks and vinblastine 6 mg/m^2 every 3 weeks (MV). A maximum of 10 cycles were recommended in responding patients while 6 cycles were foreseen in patients with stable disease. Preliminary results on 105 patients of taxotere arm and on 95 patients MV arm are available. No differences were found for the main patient characteristics between taxotere and MV patients (median age 50 (30-73) vs 50 (33-73), performance status 90 (60-100) vs 90 (60-100), previous hormonal treatment 65% vs 68%, previous response to anthracycline (not resistant 33% vs 39%; primary resistant 25% vs 19%; secondary resistant 42% vs 42%), site of disease (visceral 74% vs 73%; bone 52% vs 63%; visceral plus bone 17% vs 20%; visceral plus bone plus soft tissue 21% vs 24%), and ≥ 3 sites involved 41% vs 52%). The preliminary analysis was conducted both in all randomized patients and on eligible/evaluable patients.In the randomized population the overall response rate (ORR) is higher in taxotere group 28% with respect in MV group 13%. The same ratio of difference is confirmed in evaluable population (28% vs 14% in taxotere and MV group respectively). A CR was achieved in the 5% of patients treated with taxotere and 2% of patients of MV arm. The higher response rate was observed in patients with bidimensionally measurable disease 32% in taxotere arm whereas in MV arm no major difference with respect the ORR were observed (12%). The median number of cycles administered were 6 (range 1-12) in taxotere group and 3 (range 1-10) in MV group with a median RDI of 0.96 for taxotere patient, 0.99 for Mitomycin C and 0.98 for Vinblastine. A hundred and ninety-four patients; 104 in taxotere arm and 94 in MV arm were evaluable for safety. One patient in each group was randomized but did not receive the treatment. Ten % and the 14% of the patients discontinued the study drug for adverse events in taxotere and MV group respectively. The 7% of patients withdrew MV treatment for thrombocytopenia whereas the 6% of patients discontinued the taxotere treatment for fluid retention. Death occurred in 6.7% of patients in taxotere and

in 6.3% of patients in MV group. One septic death and 1 death due to unexplained respiratory failure occurred in taxotere group while 1 death due to hemolytic uremic syndrome occurred in MV group. One patient in each group died of unrelated event. All the other deaths were due to progressive disease. The most frequent adverse event reported in both arms was neutropenia 98% and 87.5% in taxotere and MV arms respectively. Grade 3/4 episodes occurred in 88.8% of patients treated with taxotere and in 67% of patients treated with MV. The percentage of febrile neutropenia was higher in taxotere arm (10.6%) with respect MV arm (1.1%), while thrombocytopenia was higher in MV group (35.9%) with respect taxotere group (8.7%) including grade 3/4 episodes 12% in MV vs 5.8% in taxotere group. The main grade 3 and 4 non hematological side effects experienced by patients in taxotere group versus MV group were: diarrhea 8.6% vs 0%; stomatitis 11.5% vs 1.1%; asthenia 16.3% vs 8.5%, constipation 0.9% vs 6.4%; pulmonary disorder 2.9% vs 6.4%; fluid retention 9.6% vs 0%.

Furthermore, the combination of anthracyclines and taxanes resulted in a better response rate and, in some cases, a longer time-to-progression than standard anthracycline-based regimens. Consequently, taxane-anthracycline combinations are now widely used as standard first-line treatment for advanced breast cancer.

1.3.6 Anthracycline/Docetaxel cross-resistence

In vitro, Docetaxel has been described to be recognized by the P. Glycoprotein. In addition, it has been established that docetaxel had IC50 (reducing survival by 50%) value identical on 6/11 tumor cell lines overexpressing the mdr gene. On the other hand, a docetaxel resistant human cancer cell line were isolated by Arioka. This investigator reported that no cross resistance was observed to doxorubicin and etoposide when exposing this human lung adenocarcinoma cell line to these drugs.

In vivo, in terms of patterns of cross resistance with other antitumor agents, cross resistance to docetaxel has been observed in multidrug resistant sublines such as P388/doxorubicin, CEM/VLB 1000, MCF-7/VCR GE and the CHO/CHRC5. However, these results must be looked at with caution since P388 is poorly sensitive to docetaxel and as P388 / doxorubicin and P388/VCC express high levels of mdr which may not be relevant to the clinical situation.

Recently, and in order to investigate and to better understand a possible clinical cross resistance to docetaxel, Mc Bissery has developed a docetaxel resistant mouse B16 melanoma model, after 17 months and 27 passages of repeated exposure to IV Taxotere at the maximum tolerated dose (60 mg/kg) (Bissery et al 1990). The B16/TXT melanoma was found cross resistant to vincristine and vinblastine while only partial cross resistance to doxorubicin was noted. (B16/TXT = 0.9 log cell kill and B16 = 2.4 log cell kill).

In addition, no cross resistance was observed with the other tested drugs (cyclophosphamide or etoposide).

Based on the above in vitro and in vivo data, cross resistance to PgP expressing cancer lines is partial in Taxotere resistant cell lines and in Taxotere resistant transplantable tumors and there is apparently no or only a partial cross resistance to doxorubicin.

There are several clinical data suggesting at least a partial non cross resistance for doxorubicin and docetaxel.

Three studies (two american and one european studies) were prospectively conducted in anthracycline resistant patients with metastatic disease. Their results confirm the preclinical data already reported. Two american studies (TAX 233 and TAX 267) were conducted in anthracycline/anthracenedione primary resistant (refractory) and secondary resistant patients. Overall 83 patients were recruited and 80 patients were considered resistant: 60 to doxorubicin and 20 to mitoxantrone. The third study (TAX 286) is an european study conducted in strictly defined anthracycline primary resistant (refractory) patients. Fifty-one patients were treated. In these 3 studies, the following resistance definitions were used:

- anthracycline/anthracenedione primary resistant patients were patients who progressed during an anthracenedione/anthracycline regimen or those who developed metastatic disease while still receiving adjuvant chemotherapy with anthracycline/anthracenedione containing regimen,
- or those who had an initial response and then progressed while still receiving this treatment. Those patients are considered as secondary resistant.

It is to be noted that no patients in Tax 286 experienced a response (CR or PR) to a prior anthracycline containing regimen, whereas 9 patients (15%) in Tax 233 and 267 had a prior objective response before progressing under the same anthracycline containing regimen. Among the anthracycline resistant patients, 94% in Tax 286 and 82% in Tax 233 and 267 represented the poorest population since either they did not experience any response to an anthracycline containing regimen and progressed during anthracycline or developed a relapse during an adjuvant chemotherapy. This population which has been prospectively recruited in Tax 286 and retrospectively analyzed in Tax 233 and 267 has been defined as "anthracycline refractory" to identify a subgroup with a worse prognosis.

Patients treated in Tax 286 had very aggressive disease associated with poor prognostic factors: 63% were less than 50 years of age; the median time between the first histological diagnosis and first study drug infusion was only 25 months; 67% had at least one visceral site of disease involvement (43% had liver involvement and 33% had lung involvement); 98% patients had distant metastases and only one patient had locoregional disease; 41% of patients had more than two organs involved ; and 82% had received a prior chemotherapy for metastatic disease and 49% had received 2 prior regimens of chemotherapy (one with adjuvant intent and one for metastatic disease).

The incidence of the relevant prognostic factors of patients treated in Tax 286 and in Tax 233 and 267 (table 2) are quite similar except for the age at study entry. Patients in Tax 286 were younger (62.7% < 50 years old) than those in Tax 233 + 267 (43.4% < 50 years old). In addition, 20 out of 39 patients of Tax 286 with data on receptor status were negative for both estrogen and progesterone receptors.

This last observation along with both a short median time between the first histological diagnosis and the first infusion of study drug (25 months vs 34 months in Tax 267 and 20 months in Tax 233) and a stricter definition of anthracycline resistance in Tax 286 (i.e., primary resistant or refractory patients) suggest a worse prognosis for patients treated in Tax 286 in comparison to those treated in Tax 233 and 267.

As expected, a lower response rate was observed in Tax 286 (29.4%, intent-to-treat analysis; 31.6%, evaluable patients analysis) than that observed in Tax 233 (46.3%, intent-to-treat analysis; 54.5% evaluable patients analysis) and in Tax 267 (50% and 57.1%, respectively). The difference observed could be related to the aforementioned worse prognosis of the patients recruited in Tax 286: no patients had a response (CR, PR) to a prior anthracycline containing regimen; the majority of them were < 50 years old; a considerable number of patients were negative for both estrogen and progesteron receptors; a short median interval time was detected between the first histological diagnosis and the first study drug infusion. All these factors suggest a rapid evolution of the natural history of the disease. To further support this hypothesis, it is to be noted that, in Tax 286, a higher response rate was observed in the evaluable patient subgroup $\geq$ 50 years old (46.6%) compared to the patient subpopulation aged < 50 years (21.7%).

Again a lower response rate was observed among the patients in Tax 286 when calculated by prognostic factors. However, when the results of the 3 anthracycline resistant studies were pooled, the response rates among patients with visceral metastases (43.4% out of 76 evaluable patients), liver metastases (30.7% out of 39 evaluable patients), $\geq$ 3 organs involved (48% out of 50 evaluable patients), and baseline performance status of 2 as per WHO scale (42.8% out of 14 evaluable patients) were remarkable for a single agent in an anthracycline resistant (both primary and secondary resistant) patient population.

Although all the aforementioned median times in Tax 286 are slightly shorter than those observed in Tax 233 and 267, the results of Tax 286 can still be considered remarkable for monochemotherapy in a patient population with very aggressive disease.

2 AIMS OF THE STUDY

The results of the phase III trial in advanced breast cancer prompted randomized trials designed to evaluate the effect of taxanes, combined with or in sequence with anthracycline-based regimens, in the adjuvant treatment of EBSC patients.

The Taxit 216 multicenter phase III trial was designed in 1998; we aimed to assess the efficacy and the toxicity of including docetaxel in a block-sequential fashion to a regimen with doxorubicin followed by CMF in node-positive early breast cancer patients.

In details, were selected primary and secondary objectives.

Primary objectives:

- to compare the disease free survival (DFS) in patients treated with the sequential epidoxorubicin CMF regimen to that in patients treated with the same treatment plus docetaxel given sequentially after epidoxorubicin.

Secondary objectives:

- to evaluate the overall survival in each arm;
- to compare the safety of a sequential epidoxorubicin→docetaxel→CMF (arm B) regimen versus a standard sequential epidoxorubicin→CMF (arm A).

3 MATERIALS AND METHODS

3.1 Eligibility

Eligible women were aged between 18 and 64 years and had undergone primary surgery with clear margins (modified mastectomy or tumorectomy) plus axillary dissection for histologically proven unilateral carcinoma of the breast (stage II-IIIa). Randomization was required within 6 weeks after breast cancer surgery. In the original protocol, eligible women had at least four positive axillary lymph nodes (of a minimum 10 nodes examined).

The protocol was amended one year after trial onset to allow inclusion of patients with 1–3 positive nodes and up to 70 years of age. Other eligibility criteria were baseline left ventricular ejection fraction above the lower normal limit of each participating institution and adequate hematologic (granulocyte count $\geq 2x10^9$/L, platelet count $\geq 100x10^9$/L), hepatic (transaminases $\leq$ 1.5 x the upper limit of normal [ULN], alkaline phosphatases $\leq$ 2.5 x ULN, and bilirubin $\leq$ ULN) and renal (serum creatinine $\leq$ 140 µmol/L [1.6 mg/dl] or creatinine clearance $\geq$ 60 ml/min) function.

Mayor exclusion criteria included pregnancy, documented history of cardiac disease contraindicating anthracyclines, previous cancer (except treated basal-cell and squamous-cell carcinoma of the skin or cancer of the uterine cervix), peripheral neuropathy > grade 2 according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2, and previous radiation therapy, hormone therapy, or chemotherapy for breast cancer.

Potentially eligible patients were staged by bone scan, chest x-ray, abdominal ultrasound, and contralateral mammography (Table 1).

Estrogen receptor (ER) status was evaluated by immunohistochemistry with a cut-off of 10% of stained cells.

Written informed consent was obtained before randomization.

The protocol was reviewed and approved by ethics committees and institutional review boards of each center. The study was conducted according to the Declaration of Helsinki and European Good Clinical Practice requirements.

Table 1: Prestudy screen

	INVESTIGATIONS	TIMING
History and physical exam	**Obtain patient informed consent** History -including: diagnosis of breast adenocarcinoma, prior antitumor therapy and outcome, menopausal status, general medical history, concurrent illness. Concomitant medications, prior use of medication containing polysorbate 80 (see Appendix IV) and history of allergy; medications and their indication, used within one month prior to study entry. Physical Exam - including: height and weight, WHO index for performance status, vital signs, neurologic examination	Within 7 days prior to first infusion
Hematol. *	CBC: RBC, WBC, differential count, platelets count, hemoglobin	Within 7 days prior to first infusion
Biochem. *	Alkaline phosphatase, LDH, AST (SGOT), ALT (SGPT), bilirubin, serum creatinine, creatinine clearance (if indicated), Na^+, K^+, calcium, protein, albumin, urinalysis (dipstick)	Within 7 days prior to first infusion
Radiology	• Mammograms • Chest-X-Ray (AP and lateral) with or without CT scan • Abdominal ultrasound with or without CT scan • Bone scan + bone X-rays or CT scan or MRI on hot spots observed on bone scan • Other instrumental examinations if indicated	Within 2 months prior to first infusion
ECG	ECG	Within 7 days prior to first infusion
LVEF	MUGA scan or echocardiography	Within 2 weeks prior to first infusion
Other Investigations	As clinically indicated	Within 7 days prior to first infusion

* Laboratory assessments will be performed whenever possible in the same laboratory throughout the study.

3.2 Randomization procedure and treatment protocol

Patients were allocated to treatment by an automated minimization procedure and stratified by center, number of positive lymph nodes (1–3, 4–9, 10 or more), ER status (negative, positive, unknown), and menopausal status (pre, post). Patients were assigned 1:1 to receive either epirubicin 120 mg/m² on day 1, every 21 days for 4 cycles followed by CMF 600/40/600 mg/m² on days 1 and 8, every 28 days for 4 cycles (arm A: E→CMF) or the same treatment with docetaxel 100 mg/m² on day 1, every 21 days for 4 cycles administered after the 4 cycles of epirubicin and before the 4 cycles of CMF (arm B: E→T→CMF). Therefore, patients in arm A were assigned to receive 8 cycles of chemotherapy, while patients in arm B were assigned to receive 12 cycles. Randomization was done centrally by fax at the coordinating center (University of Naples Federico II, Naples, Italy).

A third arm initially enrolled patients with four or more positive nodes at selected centers to test the feasibility and efficacy of a dose-dense regimen of 4 cycles of epirubicin 120 mg/m², day 1, every 14 days followed by 4 cycles of dose-dense docetaxel 100 mg/m², day 1, every 14 days and then 3 cycles of high-dose cyclophosphamide 3000 mg/m², day 1, every 21 days (arm C: $E_{dd}\rightarrow T_{dd}\rightarrow C_{hd}$). A feasibility analysis was planned after inclusion of the first 25 patients in arm C using pre-specified safety criteria. This analysis led to early closure of this dose-intensified arm due to unacceptable toxicity.

Docetaxel was infused over 1-hour with routine steroid premedication to prevent hypersensitivity reactions and fluid retention starting 12 hours before and ending 18 hours after the infusion. Antiemetics (5-HT3 receptor antagonists) were routinely prescribed before each cycle. Primary prophylaxis with granulocyte colony-stimulating factors (G-CSF) was mandatory for arm C, whereas it was prohibited for arms A and B. In the event of an absolute neutrophil count less than 1.5×10^9/L or platelet count less than 100×10^9/L on day 1 of each cycle, treatment was delayed until recovery. Prophylactic G-CSF was recommended for subsequent cycles in case of treatment delay due to neutropenia lasting more than 7 days or if the patient had suffered febrile neutropenia or grade 3-4 infection. If these problems persisted despite G-CSF treatment, a 25% dose-reduction was required for further chemotherapy. The same dose-reduction was required in case of a delay of more than 7 days due to thrombocytopenia, and in case of severe nonhematologic toxicity. If on day 8 of the CMF cycle, absolute neutrophil count was $< 1.0 \times 10^9$/L and/or platelets were $<100 \times 10^9$/L, chemotherapy was omitted and the subsequent cycle was started on day 21 instead of day 28.

Radiation therapy was mandatory after breast-conserving surgery and was started after completion of chemotherapy. No specific recommendations were given for post-mastectomy radiation therapy, which was according to the guidelines of each center. Tamoxifen 20 mg/day for 5 years was recommended after completion of chemotherapy for premenopausal patients with ER-positive

and unknown tumors and to all postmenopausal patients irrespective of ER status, according to current practice at the time the protocol was developed.

3.3 Statistical considerations

3.3.1 End-points

As planned in the protocol primary endpoint was Disease Free Survival (DFS) defined as the time between the date of randomization and the date of local or distant recurrence or contralateral breast cancer or second primary malignancy or death from any cause, whichever occurred first. No specification was given in the protocol as to whether consider ductal carcinoma in situ (DCIS), either contralateral or ipsilateral, as an event. Quite recently, standardized definitions for efficacy endpoints (STEEP system) in adjuvant breast cancer trials have been proposed by a multidisciplinary panel of experts with the aim of reducing inconsistencies of results across clinical trials (Hudis et al 2007).

Thus we decided to report the results according to such a system.

Primary end-point was accordingly re-defined Invasive-DFS (IDFS), which excluded DCIS from the events of interest. Efficacy results were also provided for Overall Survival (OS), and Recurrence-free survival (RFS). OS was defined as the time from randomization to death from any cause. RFS was defined as the time between the date of randomization and the date of local or distant recurrence or death from any cause, whichever occurred first; thus contralaeral breast cancer or second primary (non-breast) cancers were excluded (Hudis et al 2007).

3.3.2 Sample size calculation

The trial was originally designed to enrol 752 patients, 732 for the comparison of arm A *vs* arm B plus 120 patients for the comparison of arm B *vs* arm C. This was based on an expected 5yr DFS equal to 0.55 in arm A (Bonadonna et al 1995), a 10% absolute improvement in arm B and a further 15% absolute gain in arm C, with a type I error of 0.05 (two sided) and a power of 80%. In June 1999 inclusion criteria were amended, allowing the inclusion of subjects with 1 to 3 axillary metastases, and sample size was re-estimated accordingly leading to a total sample size of 914 patients (794 for the comparison of arm A *vs* arm B plus 120 patients for the comparison of arm B *vs* arm C).

Upon closure of arm C for toxicity the Steering Committee decided to re-evaluate again the sample size of the study to possibly increase the power of the first comparison. For this final calculation, expected 5yr DFS in arm A was set to 0.65 based on the results, which had become available meanwhile, of a

previous trial evaluating the efficacy of a block-sequential anthracycline-CMF regimen in node-positive patients (De Placido et al 2005).

Assuming an absolute improvement for the experimental arm (arm B) of 9% (HR equal to 0.70), a type I error (α) equal to 0.05 (two sided) and a power of 80%, it was estimated that a total of 960 subjects and 250 events would be needed for the final analysis.

All sample size adjustments were done blinded to data.

3.3.3 Statistical analysis

All efficacy analysis were done on an intention-to-treat basis. All subjects receiving at least one treatment dose were considered evaluable for efficacy analysis.

Time-to-event curves were estimated with Kaplan-Meier (K-M) product limit, and statistical significance was assessed with a 2-sided log-rank test. Adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated using a Cox proportional hazards model that included treatment, lymph node metastases (1-3, 4-9, 10+), ER status (negative, positive, unknown) and menopausal status (pre/post) as covariates.

Exploratory subgroup analysis were reported as 'Forest plot', with 95% confidence intervals, focusing on possible interactions with treatment rather than strictly relying on statistical significance.

All patients who received treatment were considered for toxicity analysis. Up to six laboratory exams were planned for each cycle. Statistical analysis of toxicity was done in two ways. First, an exact linear permutation test was applied to allow for the ordinal nature of toxicity grades (Cytel 7 software, Cambridge, MA). Second, an exact chi-square test was applied comparing severe (grades 3 to 4) versus non-severe (grades 0 to 2) toxicity.

Compliance to treatment was reported both on a per patient and a per cycle basis, according to treatment actually received. This analysis was descriptive only.

All the analysis were performed with SAS version 8.2 (SAS Inc., Cary, NC, USA) and graphs were made with R 2.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

4 RESULTS AND DISCUSSION

4.1 Patients

From July 1998 to July 2002, 998 patients were randomized in to the trial (arm A: n=486; arm B: n=486; arm C: n=26). After inclusion of 26 patients in arm C, a planned interim safety analysis was conducted to evaluate the feasibility of the dose-intensified treatment. All feasibility rules were met except for grade 4 skin toxicity in one patient, which led to early closure of arm C, for which no further results are reported. Baseline characteristics of all randomized patients were well balanced (Table 2). Median age was 51 years (range: 23–74). The study included a similar number of pre- and post-menopausal women. Two-thirds of tumors were ER-positive.

Table 2: Patient characteristics by treatment arm*

		E → CMF (Total 486) No. (%)	**E → T →CMF (Total 486) No. (%)**
Age	<50	227 (46.7)	213 (43.8)
	≥50	259 (53.3)	273 (56.2)
Menopause	Pre	244 (50.2)	243 (50.0)
	Post	242 (49.8)	243 (50.0)
TNM	T1	194 (39.9)	219 (45.1)
	T2	242 (49.8)	203 (41.8)
	T3-4	50 (10.3)	64 (13.2)
Nodes (No.)	1-3	179 (36.8)	178 (36.6)
	4-9	193 (39.7)	198 (40.7)
	>=10	114 (23.5)	110 (22.6)
Histology	Ductal	390 (80.3)	378 (77.8)
	Lobular	59 (12.1)	71 (14.6)
	Other	37 (7.6)	37 (7.6)
ER	Negative	114 (23.5)	117 (24.1)
	Positive	319 (65.6)	315 (64.8)
	Unknown	53 (10.9)	54 (11.1)

* E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil; TNM = tumor, node, metastasis; ER = estrogen receptor

4.2 Adherence to treatment

The planned number of chemotherapy cycles was administered to 91% patients in arm A and 74% in arm B. Reasons for treatment discontinuation are shown in Table 3. Overall, 96% and 93% of patients received four planned cycles of epirubicin and CMF, respectively, at a rate that was similar between arms, and 83% of patients received 4 cycles of docetaxel.

Overall, chemotherapy was delayed in 18% of cycles in arm A and 20% of cycles in arm B. Dose reduction was applied in 4% of cycles in arm A *vs* 7% in arm B. Docetaxel administration was delayed in 21% of cycles and the dose was reduced in 11% of cycles.

Table3: Reasons for discontinuation of chemotherapy by received treatment*

Reasons	**E →CMF**	**E → T →CMF**
Breast cancer relapse	3	7
Second primary malignancy	0	1
Adverse experience	17	31
Consent withdrawal	12	43
Death	0	1
Protocol deviation	0	2
Lost	0	3
Other	4	25
Not Reported	10	14
Total	46	127

* E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil**;** E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil

4.3 Efficacy

At the date of this analysis (November 30, 2006), 142 patients had died and the median follow-up was 62 months. A total of 278 IDFS events had occurred (Table 4).

Table 4: Patients with first IDFS events*

	E →CMF (total 486) No. (%)	E → T →CMF (total 486) No. (%)
Breast cancer relapse	139 (28.6)	108 (22.2)
Loco/regional	25 (5.1)	19 (3.9)
Distant	114 (23.5)	89 (18.3)
Death	5 (1.0)	7 (1.4)
Second primary cancer	5 (1.0)	14 (2.9)
Breast	1 (0.2)	3 (0.6)
Other	4 (0.8)	11 (2.3)
Total IDFS events	149 (30.7)	129 (26.5)
None (event-free patients)	337 (69.3)	357 (73.5)

* E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil**;** E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil; IDFS = invasive disease free survival.

IDFS was better in the experimental arm (B), although the difference did not reach statistical significance (log-rank $P = 0.134$) (Figure 1). The estimated probability of being free of any IDFS event at 5 years was 74% for arm B and 68% for arm A (HR 0.82, 95% CI 0.64-1.03; $P = 0.1337$).

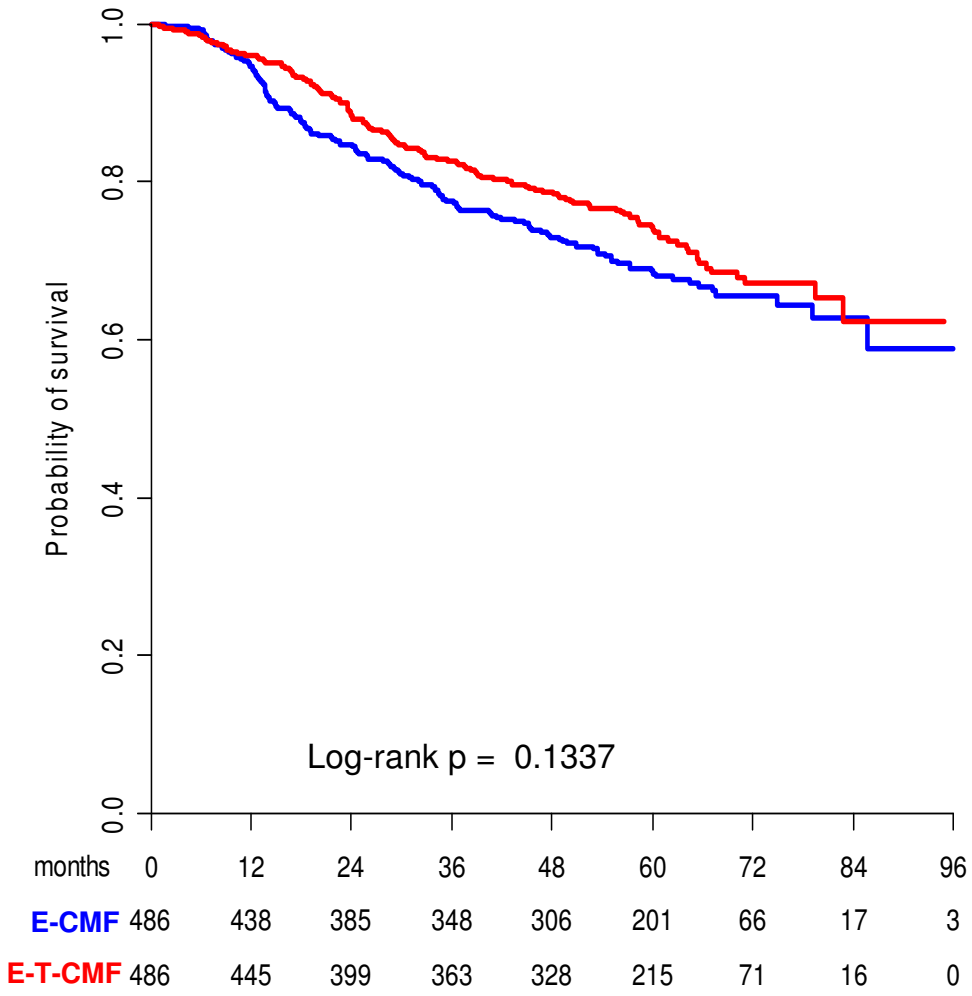


Figure 1: IDFS for arm A and for arm B. E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil; E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil.

The estimated probability of being recurrence-free at five years was significantly better for arm B than for arm A (76% *vs* 69%, respectively; HR 0.75; 95% CI 0.59-0.96; *P* = 0.0332; Figure 2).

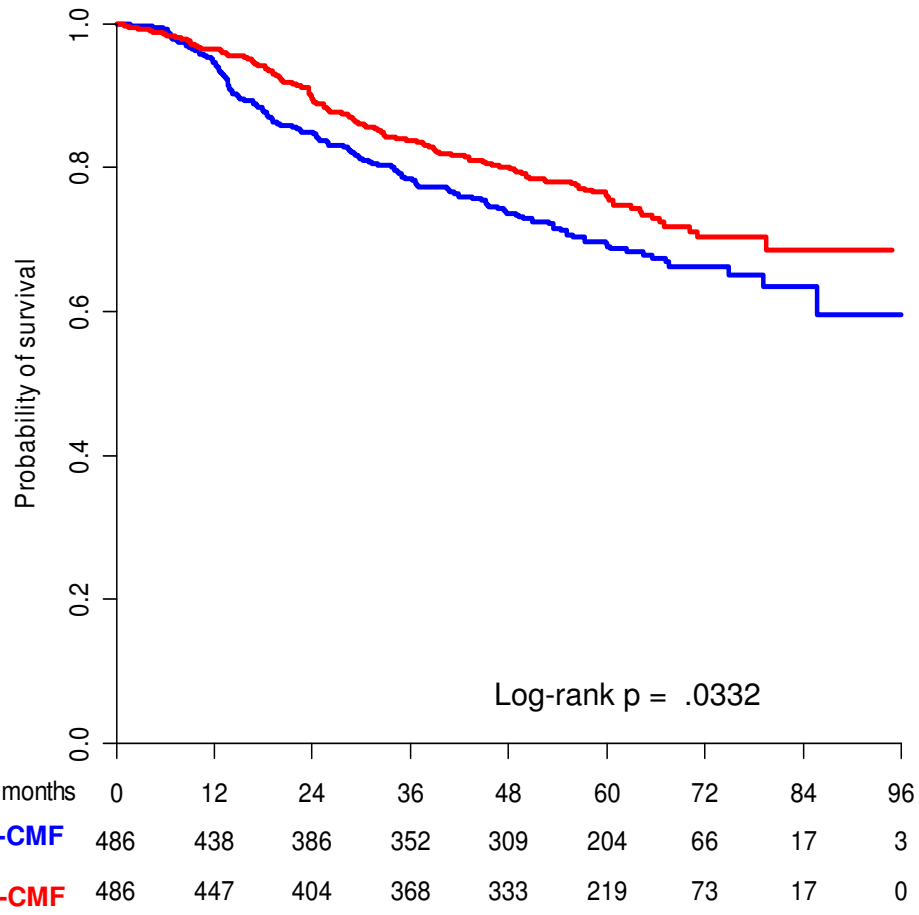


Figure 2: RFS for arm A and for arm B. E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil; E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil.

A statistically significant improvement was observed for OS (Figure 3), with an estimated probability of being alive at five years of 90% for arm B and 85% for arm A (HR 0.67; 95% CI 0.48-0.94; *P* = 0.0168).

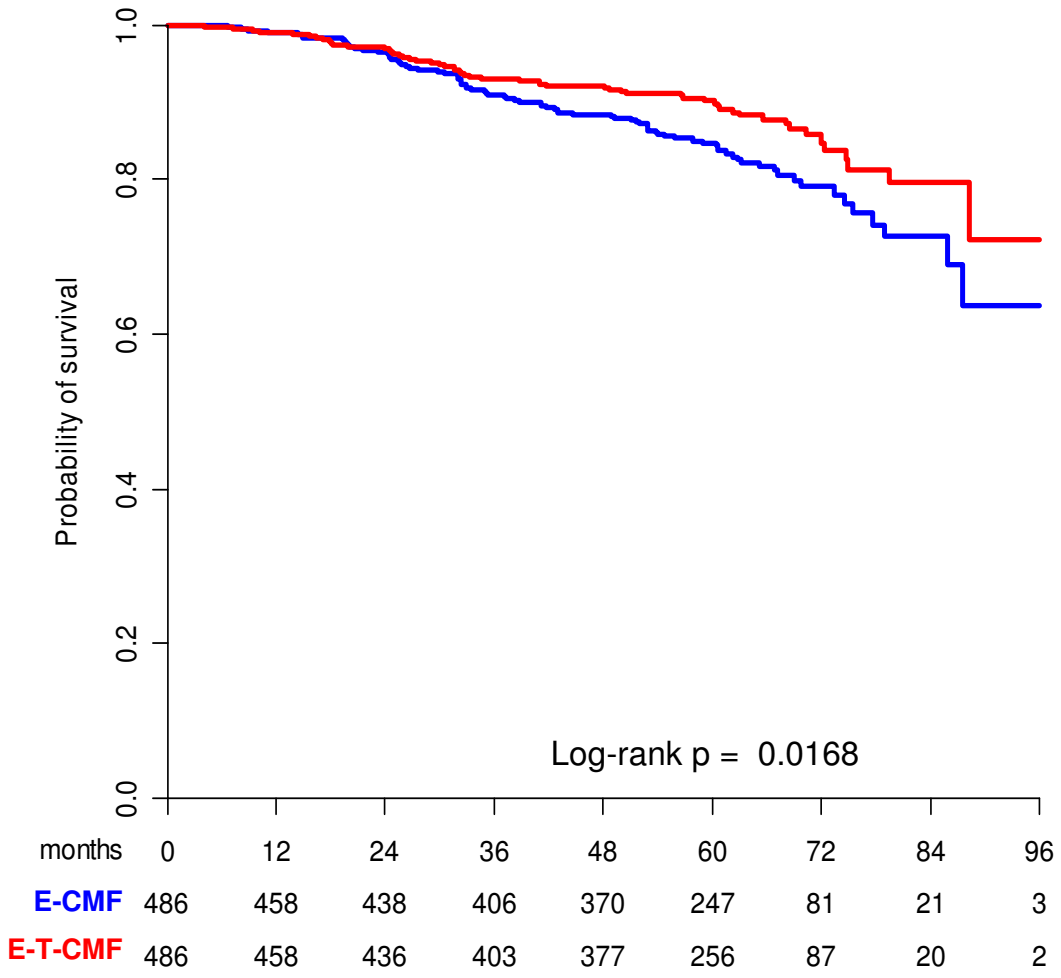


Figure 3: OS for arm A and for arm B. E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil; E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil.

Multivariate analysis that included treatment, lymph node metastases, ER status and menopausal status as covariates confirmed these results. There was no evidence of heterogeneity of treatment efficacy in the subgroups of patients stratified according to age, menopausal status, ER status and number of positive lymph nodes (Figure 4).

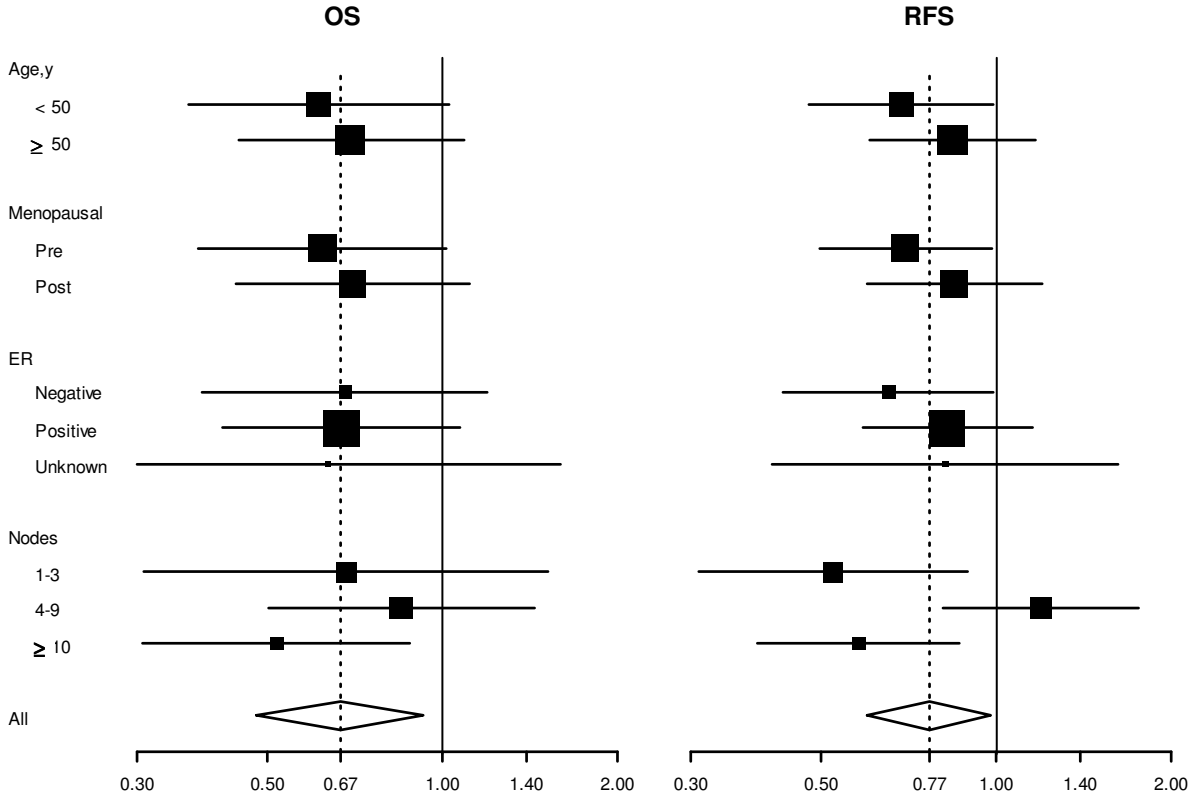


Figure 4: Forest Plot of Overall Survival (OS) and Relapse Free Survival (RFS) Hazard Ratios of main subgroups (exploratory analysis).

4.4 Toxicity

In both arms, grade 3-4 neutropenia rates were higher than usually reported (84% and 90% in arms A and B, respectively; $P = 0.009$). The rate of febrile neutropenia was significantly higher in the experimental arm (11.6% *vs* 6.2%). When the analysis of neutropenia was limited to laboratory values recorded the day before staring a new cycle (day 21 for epirubicin and docetaxel; day 28 for CMF), grade 3-4 neutropenia rates decreased to 10% and 12% for arm A and arm B, respectively ($P = 0.42$). Severe anemia and thrombocytopenia were uncommon (Table 5).

Table 5: Hematological toxicity: grade 3-4 events according to treatment arm*

	E→CMF No. (%)	**E→T→CMF No. (%)**	***P*†**
Neutropenia at nadir	406 (84.4)	434 (90.2)	0.0087
Neutropenia at recycling	50 (10.4)	59 (12.4)	0.4158
Anemia	10 (2.1)	12 (2.5)	0.6739
Thrombocytopenia	11 (2.3)	11 (2.3)	0.9999
Leucopenia	244 (50.6)	315 (65.5)	<0.0001
Febrile neutropenia	30 (6.2)	56 (11.6)	0.0032

*E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil; E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil;

†Fisher's exact test comparing grade 0-1-2 *vs* 3-4

Grade 3–4 nonhematologic adverse events are reported in Table 6. More patients in arm B experienced at least one nonhematologic event. Several events were reported only for arm B: neurotoxicity in 1% of patients; hypersensitivity in 1.6% of patients; and peripheral edema in <1% or patients. Other grade 3–4 toxicities that were more frequent among docetaxel patients included: asthenia, arthralga, myalgia (none in control arm), diarrhea, stomatitis, and skin and nail disorders (none in control arm). Cardiac function toxicity occurred in only in arm B (1 patient; 0.2%), whereas cardiac ischemia occurred only in arm A (1 patient; 0.2%).

Overall, 5 non-breast cancers were reported in arm A: small-cell lung (n=1), thyroid (n=1), pancreatic (n=1), ovarian (n=1), and unspecified uterine (n=1). Twelve non-breast cancers occurred in arm B: colon (n=2), thyroid (n=2), melanoma (n=1), kidney (n=1), tonsil (n=1), PNET (n=1), ovarian (n=1), endometrial (n=1), 1 uterine cervix (n=1), and 1 unknown (n=1). Neither acute myeloid leukemia nor myelodysplastic syndrome has been recorded to date.

Table 6: Nonhematological toxicity (grade 3-4 events) according to treatment arm*

	E → CMF No. (%)	**E → T →CMF No. (%)**	***P*†**
Allergy	0 (0.0)	8 (1.6)	0.0037
Arthralgy	1 (0.2)	6 (1.2)	0.0689
Asthenia	12 (2.5)	31 (6.4)	0.0029
Cardiac arrhythmias	0 (0.0)	0 (0.0)	0.9999
Cardiac function	0 (0.0)	1 (0.2)	0.499
Cardiac ischemia	1 (0.2)	0 (0.0)	0.9999
Cardiac pericardial	0 (0.0)	0 (0.0)	0.9999
Diarrhea	5 (1.0)	22 (4.5)	<0.0001
Local toxicity	2 (0.4)	3 (0.6)	0.6862
Myalgia	0 (0.0)	7 (1.5)	0.0075
Nail disorders	0 (0.0)	8 (1.7)	0.0037
Nausea	23 (4.7)	34 (7.0)	0.1353
Neuromotor	0 (0.0)	5 (1.0)	0.0306
Neurosensory	0 (0.0)	5 (1.0)	0.0306
Pain	0 (0.0)	4 (0.8)	0.0616
Peripheral edema	0 (0.0)	3 (0.6)	0.1238
Pulmonary	2 (0.4)	2 (0.4)	0.9999
Skin	0 (0.0)	15 (3.1)	<0.0001
Stomatitis	30 (6.2)	46 (9.5)	0.0565
Vomiting	25 (5.2)	35 (7.2)	0.1851
Weight gain	1 (0.2)	1 (0.2)	0.9999

*E→CMF = epirubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil**;** E→T→CMF = epirubicin followed by docetaxel followed by cyclophosphamide, methotrexate, and 5-fluorouracil;

†Fisher's exact test comparing grade 0-1-2 *vs* 3-4

4.5 Discussion

The TAXIT 216 trial shows that the addition of four cycles of docetaxel to a block-sequential epirubicin-CMF regimen reduces the risk of recurrence and death in patients with lymph-node positive ESBC.

Although the reduction in risk recurrence did not reach statistical significance for IDFS (the primary end point), it was statistically significant for RFS. This apparent discrepancy is probably due to the inclusion of non-breast second primary tumors among the events used to estimate IDFS as required by STEEP guidelines (Hudis et al 2007). Although inclusion of non-breast cancer events in the analysis of IDFS avoids misdiagnosis of a distant recurrence as a second primary cancer, it dilutes any treatment effect and so, these second primaries, should not be related to the treatment under study.

In our trial, there was a small, not statistically significant, excess of non-breast second primary tumors in the experimental arm. However, no evidence of excess non-breast cancer events in taxane-based arms emerged from any of the randomized trials of taxane-based adjuvant regimens published to date. Furthermore, the incidence of non-breast primaries in our experimental arm is around the average of other trials, whereas incidence was very low in our control arm (Table 7). These observations suggest that the imbalance of non-breast cancer events in the TAXIT 216 trial is probably unrelated to treatment and occurred by chance.

Consequently, their inclusion in the analysis of the primary end point may have weakened the power of the comparison. In this situation, a better estimate of the therapeutic effect may be RFS, which in our trial was significantly improved by the addition of docetaxel (25% relative risk reduction; $P = .0332$). Consistent with this outcome, there was also a statistically significant advantage in OS (33% relative risk reduction; $P = .0168$) in the experimental arm.

Several phase III randomized trials have evaluated the effect of taxanes, combined or in sequence with anthracycline-based regimens, in the adjuvant treatment of ESBC patients. Most, but not all, the efficacy data available show a significant reduction of the risk of recurrence for the taxane-based treatment versus the control anthracycline-based regimen (Budzar et al 2002, Martin et al 2005, Mamounas et al 2005, Evans et al 2005, Gianni et al 2005, Jones et al 2006, Bear et al 2006, Martin et al 2008, Francis et al 2008, Goldestein et al 2008). On the other hand, a benefit in OS has been found only in a few trials (Henderson et al 2003, Fountzilas et al 2005, Martin et al 2005, Roche et al 2006). However, a recent meta-analysis of all available randomized trials suggests that, on average, the addition of a taxane to anthracycline-based treatment yields significant benefits in terms of OS (De Laurentiis et al 2008). The final analysis of our trial is consistent with this finding, and lends support to the use of taxanes, particularly docetaxel, in the adjuvant setting.

Table 7: Non-breast primaries in published adjuvant taxane trials

	Non-Breast Cancers reported as first event	
	Taxane No. (%)	**No Taxane No. (%)**
Paclitaxel-based Trials		
M.D.ANDERSON (MDACC2002)	NR	NR
CALGB 9344	NR	NR
GEICAM 9906	15 (2.4)	12 (1.9)
HeCOG 10/97	3 (1.0)*	4 (1.3)*
NSABP B28	36 (2.1)	50 (2.9)
Docetaxel-based Trials		
BCIRG 001	13 (1.7)	18 (2.4)
NSABP B27	43 (2.7)	17 (2.1)
Anglo-Celtic	NR	NR
BIG 2-98	32 (1.7)	18 (1.9)
PACS 01	17 (1.7)*	25 (2.5)*
ECOG E 2197	57 (3.9)*	39 (2.6)*
USON	NR	NR
Taxit 216	11 (2.3)	4 (0.8)

* Events not used to estimate primary end-point; NR: not reported

Various anthracycline-based regimens have been used as control in adjuvant taxane trials. However, there is some controversy as to whether the anthracyline-based control arms in such trials could be considered standard. Indeed, not all anthracycline-based regimens used as control arms in such trials have clearly proven superiority versus CMF, thus confounding the interpretation of results.

In such a situation, clinical preference should be given to taxane-based regimens that have been compared to an anthracycline-based control. The block-sequential E–CMF regimen used as control arm in the TAXIT 216 trial is among the few control regimens in adjuvant taxane trials, if not the only one, that has bested CMF in direct randomized comparisons (De Placido et al 2005, Poole et al 2006). A similar block-sequential regimen, but with a lower number of cycles, was the control arm in the recently published BIG2-98 trial (Francis et al 2008), has yielded comparable results.

A crucial issue is whether taxanes should be combined with anthracyclines or whether they should be administered sequentially after an anthracycline-based regimen. Both options have theoretical advantages and drawbacks: combination regimens may require dose reductions for both compounds but, in theory, can exploit drug synergism; on the other hand, in sequential regimens both compounds can be administered at optimal doses.

Three decades ago, Norton and Simon reported that the growth of solid tumors could be described by Gompertzian kinetics, in which the rate of regrowth of a tumor increases as the tumor shrinks in response to therapy. The Norton–Simon hypothesis predicts that this resistance might be overcome by switching from initial chemotherapy agents to newer agents at the maximally tolerated dose. The results of the TAXIT 216 trial are consistent with this hypothesis.

However, similar results have been obtained with regimens in which the taxane was given in combination with the anthracycline. De Laurentiis and colleagues, in their meta-analysis of randomized trials (De Laurentiis et al 2008), made an exploratory indirect comparison between block-sequential and combination regimens and did not find a statistically significant difference in efficacy. However, because of the indirect nature of this comparison, we cannot exclude that there could be moderate but meaningful differences in efficacy between sequential and combination regimens. The only trial reporting a direct randomized comparison between a sequential and combination taxane-based regimen is the BIG2-98 trial, which showed an advantage for the sequential regimen of borderline statistical significance.

However, caution should be exerted in drawing conclusions about this issue based on the BIG2-98 and TAXIT 216 trials because of design issues (ie, the longer treatment duration of the experimental arm, the different docetaxel/anthracycline doses, the unplanned nature of this comparison). Results of other adjuvant trials, such as BCIRG 005 and NSABP B-30, comparing sequential and concurrent anthracycline-docetaxel regimens, are eagerly awaited.

There is some controversy about whether taxanes produce consistent benefit across specific subgroups of patients. Data from individual trials suggest that the benefits of taxanes may be lower, if not negligible, for patients with 4 or more positive nodes or ER-positive tumors. Other trials suggest that the benefit differs between younger and older patients.

In our trial, docetaxel reduced the risk of recurrence irrespective of ER status (positive *vs* negative), nodal status (1-3 *vs* 4-9 *vs* 10+), age (≤50 *vs* >50 years) and menopausal status (pre *vs* post). Although the relative benefit varies across some subgroups of patients (Figure 4), there is no statistically significant heterogeneity indicating that this arose by chance. Therefore, our study indicates that the number of positive lymph nodes, age, menopausal status and ER status should not be used in clinical practice to identify patients who may (or may not) benefit from our experimental regimen. The same conclusion emerges from a recent meta-analysis of randomized trials.

Two limitations of our trials deserve discussion. First, compared with similar trials, TAXIT 216 has a smaller than average sample size. It may be argued that this decreases the value of the trial results. However, underpowered trials have by definition a high chance of not detecting a statistically significant difference between treatments (ie, a high chance of being false-negative). Conversely, once a statistically significant difference is detected, as in the case of our trial, the small sample size does not decrease the absolute strength of such evidence, although it affects the precision of the point estimate for the HR. Second, our experimental treatment lasted longer than other taxane-based regimens and this may raise concerns of excess toxicity and discomfort for patients.

In this regard, our toxicity profile appears reassuring. Hematologic severe adverse events were rare in our experimental arm except for grade 3-4 neutropenia.

The high incidence of neutropenia was probably due to large number of laboratory examinations (up to six) that were required by the protocol for each cycle of chemotherapy thereby increasing the change of recording the neutropenic nadir.

In fact, when we limited the analysis of adverse events to the day planned for chemotherapy administration, the rate of severe neutropenia was similar to that of the control arm and was lower to that generally reported for shorter-lasting taxanes-based regimens.

Febrile neutropenia affected more patients in the experimental arm than in the control arm (11.6% *vs* 6.2%), but its frequency was similar to that reported for shorter block-sequential regimens (Roche et al 2006, Martin et al 2008) and less than the 25% reported for the docetaxel, doxorubicin cyclophosphamide (TAC) regimen (Martin et al 2005).

Among the other taxane-specific adverse events, severe neurotoxicity was rare (1%) and less frequent than in shorter paclitaxel-based regimens (Martin et al 2008, Mamounas et al 2005, Henderson et al 2003, Citron et al 2003, Sparano et al 2008).

Furthermore, recent data indicate that for patients, the duration of an adjuvant regimen is less important than expected and most are willing to accept a longer-lasting treatment if it is associated with a marginal additional benefit (Duric et al 2008).

5 CONCLUSIONS

Adjuvant chemotherapy, significantly, decreases the risk of recurrence and death in women with node-positive operable breast cancer. CMF regimens first and anthracycline-based combinations later, became the standard treatment for operable breast cancer.

In the 1990s, the taxanes, docetaxel and paclitaxel, were incorporated to the standard armamentarium for metastatic breast cancer. The taxanes are partially non cross-resistent with anthracyclines and, therefore, several regimens using taxanes and anthracyclines (either in combination or in sequence) were tested in the adjuvant setting.

In this context, the TAXIT 216 trial was designed to assess the efficacy of adding docetaxel in a sequential anthracycline-based regimen; with a median follow-up of 62 months, our study is the only one to show an overall survival benefit compared with an adeguate anthracycline regimen.

In conclusion, our data suggest that including docetaxel into a block-sequential epirubicin-CMF regimen significantly reduces the risk of relapse and death for node positive early stage breast cancer.

This benefit is independent of age, menopausal status, ER status and nodal status. This advantage comes at the cost of an increased, but, acceptable, toxicity.

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7 REFERENCES

Bear HD, Anderson S, Smith RE, Geyer CE, Jr., Mamounas EP, Fisher B, Brown AM, Robidoux A, Margolese R, Kahlenberg MS, Paik S, Soran A, Wickerham DL, Wolmark N. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer:National Surgical Adjuvant Breast and Bowel Project Protocol B-27. Journal of Clinical Oncology 2006; 24:2019-27.

Bissery MC, Bayssas M, Lavelle F. Preclinical evaluation of intravenous Taxotere (RP56976, NSC 628503) a taxol analog. Proc Am Assoc Cancer Res 1990;31:417.

Bonadonna G, Zambetti M, Valagussa P. Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. JAMA 1995;273:542-547.

Buzdar AU, Singletary SE, Valero V, Booser DJ, Ibrahim NK, Rahman Z, Theriault RL, Walters R, Rivera E, Smith TL, Holmes FA, Hoy E, Frye DK, Manuel N, Kau SW, McNeese MD, Strom E, Thomas E, Hunt K, Ames F, Berry D, Hortobagyi GN. Evaluation of paclitaxel in adjuvant chemotherapy for patients with operable breast cancer: preliminary data of a prospective randomized trial. Clinical Cancer Research 2002; 8:1073-9.

Buzzoni R, Bonadonna G, Valagussa P, Zambetti M. Adjuvant chemotherapy with doxorubicin plus cyclophosphamide, methotrexate and fluorouracil in the treatment of resectable breast cancer with more than three positive axillary nodes. Journal of Clinical Oncology 1991; 9:2134-2140.

Chan S, Friedrichs K, Noel D, Pinter T, Van Belle S, Vorobiof D, Duarte R, Gil Gil M, Bodrogi I, Murray E, Yelle L, Von Minckwitz G, Korec S, Simmonds P, Buzzi F, Gonzalez Mancha R, Richardson G, Walpole E, Ronzoni M, Murawsky M, Alakl M, Riva A, Crown J. Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer. Journal of Clinical Oncology 1999;17:2341-2354.

Citron ML, Berry DA, Cirrincione C, Hudis C, Winer EP, Gradishar WJ, Davidson NE, Martino S, Livingston R, Ingle JN, Perez EA, Carpenter J, Hurd D, Holland JF, Smith BL, Sartor CI, Leung EH, Abrams J, Schilsky RL, Muss HB, Norton L. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. Journal of Clinical Oncology 2003; 21:1431-9.

De Laurentiis M, Cancello G, D'Agostino D, Giuliano M, Giordano A, Montagna E, Lauria R, Forestieri V, Esposito A, Silvestro L, Pennacchio R, Criscitiello C, Montanino A, Limite G, Bianco AR, De Placido S. Taxane-based combinations as adjuvant chemotherapy of early breast cancer: a meta-analysis of randomized trials. Journal of Clinical Oncology 2008;26:44-53.

De Placido S, De Laurentiis M, De Lena M, Lorusso V, Paradiso A, D'Aprile M, Pistillucci G, Farris A, Sarobba MG, Palazzo S, Manzione L, Adamo V, Palmeri S, Ferrau F, Lauria R, Pagliarulo C, Petrella G, Limite G, Costanzo R, Bianco AR. A randomised factorial trial of sequential doxorubicin and CMF vs CMF and chemotherapy alone vs chemotherapy followed by goserelin plus tamoxifen as adjuvant treatment of node-positive breast cancer. Br J Cancer 2005; 92:467-74.

Duric VM, Butow PN, Sharpe L, Heritier S, Boyle F, Beith J, Wilcken NR, Coates AS, Simes RJ, Stockler MR. Comparing patients' and their partners' preferences for adjuvant chemotherapy in early breast cancer. Patient Educ Couns 2008; 72:239-45.

Early Breast Cancer Trialists'Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. Lancet 1998;352:930-942.

Evans TR, Yellowlees A, Foster E, Earl H, Cameron DA, Hutcheon AW, Coleman RE, Perren T, Gallagher CJ, Quigley M, Crown J, Jones AL, Highley M, Leonard RC, Mansi JL. Phase III randomized trial of doxorubicin and docetaxel versus doxorubicin and cyclophosphamide as primary medical therapy in women with breast cancer: an anglo-celtic cooperative oncology group study. Journal of Clinical Oncology 2005; 23:2988-95.

Fountzilas G, Skarlos D, Dafni U, Gogas H, Briasoulis E, Pectasides D, Papadimitriou C, Markopoulos C, Polychronis A, Kalofonos HP, Siafaka V, Kosmidis P, Timotheadou E, Tsavdaridis D, Bafaloukos D, Papakostas P, Razis E, Makrantonakis P, Aravantinos G, Christodoulou C, Dimopoulos AM. Postoperative dose-dense sequential chemotherapy with epirubicin, followed by CMF with or without paclitaxel, in patients with high-risk operable breast cancer: a randomized phase III study conducted by the Hellenic Cooperative Oncology Group. Ann Oncol 2005; 16:1762-71.

Francis P, Crown J, Di Leo A, Buyse M, Balil A, Andersson M, Nordenskjold B, Lang I, Jakesz R, Vorobiof D, Gutierrez J, van Hazel G, Dolci S, Jamin S, Bendahmane B, Gelber RD, Goldhirsch A, Castiglione-Gertsch M, Piccart-Gebhart M. Adjuvant chemotherapy with sequential or concurrent

anthracycline and docetaxel: Breast International Group 02-98 randomized trial. J Natl Cancer Inst 2008; 100:121-33.

Gianni L, Baselga J, Eiermann W. European Cooperative Trial in Operable Breast Cancer (ECTO): Improved freedom from progression (FFP) from adding paclitaxel (T) to doxorubicin (A) followed by cyclophosphamide methotrexate and fluorouracil (CMF). (Abs#513). Journal of Clinical Oncology 2005; 2005 ASCO Annual Meeting Proceedings. Vol 23, No. 16S:513.

Goldstein LJ, O'Neill A, Sparano JA, Perez EA, Shulman LN, Martino S, Davidson NE. Concurrent Doxorubicin Plus Docetaxel Is Not More Effective Than Concurrent Doxorubicin Plus Cyclophosphamide in Operable Breast Cancer With 0 to 3 Positive Axillary Nodes: North American Breast Cancer Intergroup Trial E 2197. Journal of Clinical Oncology 2008.

Henderson IC, Berry DA, Demetri GD, Cirrincione CT, Goldstein LJ, Martino S, Ingle JN, Cooper MR, Hayes DF, Tkaczuk KH, Fleming G, Holland JF, Duggan DB, Carpenter JT, Frei E, III, Schilsky RL, Wood WC, Muss HB, Norton L. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. Journal of Clinical Oncology 2003; 21:976-83.

Holmes FA, Walters RS, Theriault RL. Phase II trial of taxol an active drug in the treatment of metastatic breast cancer. J Natl Cancer Inst 1991;83:1797-1805.

Hudis CA, Barlow WE, Costantino JP, Gray RJ, Pritchard KI, Chapman JA, Sparano JA, Hunsberger S, Enos RA, Gelber RD, Zujewski JA. Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. Journal of Clinical Oncology 2007; 25:2127-32.

Jones SE, Savin MA, Holmes FA, O'Shaughnessy JA, Blum JL, Vukelja S, McIntyre KJ, Pippen JE, Bordelon JH, Kirby R, Sandbach J, Hyman WJ, Khandelwal P, Negron AG, Richards DA, Anthony SP, Mennel RG, Boehm KA, Meyer WG, Asmar L. Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. Journal of Clinical Oncology 2006; 24:5381-7.

Mamounas EP, Bryant J, Lembersky B, Fehrenbacher L, Sedlacek SM, Fisher B, Wickerham DL, Yothers G, Soran A, Wolmark N. Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-

positive breast cancer: results from NSABP B-28. Journal of Clinical Oncology 2005; 23:3686-96.

Martin M, Pienkowski T, Mackey J, Pawlicki M, Guastalla JP, Weaver C, Tomiak E, Al Tweigeri T, Chap L, Juhos E, Guevin R, Howell A, Fornander T, Hainsworth J, Coleman R, Vinholes J, Modiano M, Pinter T, Tang SC, Colwell B, Prady C, Provencher L, Walde D, Rodriguez-Lescure A, Hugh J, Loret C, Rupin M, Blitz S, Jacobs P, Murawsky M, Riva A, Vogel C. Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 2005; 352:2302-13.

Martin M, Rodriguez-Lescure A, Ruiz A, Alba E, Calvo L, Ruiz-Borrego M, Munarriz B, Rodriguez CA, Crespo C, de Alava E, Lopez Garcia-Asenjo JA, Guitian MD, Almenar S, Gonzalez-Palacios JF, Vera F, Palacios J, Ramos M, Gracia Marco JM, Lluch A, Alvarez I, Segui MA, Mayordomo JI, Anton A, Baena JM, Plazaola A, Modolell A, Pelegri A, Mel JR, Aranda E, Adrover E, Alvarez JV, Garcia Puche JL, Sanchez-Rovira P, Gonzalez S, Lopez-Vega JM. Randomized phase 3 trial of fluorouracil, epirubicin, and cyclophosphamide alone or followed by Paclitaxel for early breast cancer. J Natl Cancer Inst 2008; 100:805-14.

Nabholtz JM, Senn HJ, Bezwoda WR, Melnychuk D, Deschenes L, Douma J, Vandenberg TA, Rapoport B, Rosso R, Trillet-Lenoir V, Drbal J, Molino A, Nortier JW, Richel DJ, Nagykalnai T, Siedlecki P, Wilking N, Genot JY, Hupperets PS, Pannuti F, Skarlos D, Tomiak EM, Murawsky M, Alakl M, Aapro M. Prospective randomized trial of docetaxel versus mitomycin plus vinblastine in patients with metastatic breast cancer progressing despite previous anthracycline-containing chemotherapy. 304 Study Group. Journal of Clinical Oncology 1999; 17:1413-24.

Norton L, Simon R. The Norton-Simon hypotesis revisited. Cancer Treat Rep 1986;70:163-169.

Paridaens R, Biganzoli L, Bruning P, Klijn JCM, Gamucci T, Houston S, Coleman R, Schachter J, Van Vreckem A, Sylvester R, Awada A, Wildiers J, Piccart M. Paclitaxel versus doxorubicin as first-line single-agent chemotherapy for metastatic breast cancer: A European Organization for Research and Treatment of Cancer Randomized Study with cross-over. Journal of Clinical Oncology 2000;18:724-733.

Poole CJ, Earl HM, Hiller L, Dunn JA, Bathers S, Grieve RJ, Spooner DA, Agrawal RK, Fernando IN, Brunt AM, O'Reilly SM, Crawford SM, Rea DW, Simmonds P, Mansi JL, Stanley A, Harvey P, McAdam K, Foster L, Leonard RC, Twelves CJ. Epirubicin and cyclophosphamide, methotrexate,

and fluorouracil as adjuvant therapy for early breast cancer. N Engl J Med 2006; 355:1851-62.

Ravdin PM, Burris HA, III, Cook G, Eisenberg P, Kane M, Bierman WA, Mortimer J, Genevois E, Bellet RE. Phase II trial of docetaxel in advanced anthracycline-resistant or anthracenedione-resistant breast cancer. Journal of Clinical Oncology 1995; 13:2879-85.

Roche H, Fumoleau P, Spielmann M, Canon JL, Delozier T, Serin D, Symann M, Kerbrat P, Soulie P, Eichler F, Viens P, Monnier A, Vindevoghel A, Campone M, Goudier MJ, Bonneterre J, Ferrero JM, Martin AL, Geneve J, Asselain B. Sequential adjuvant epirubicin-based and docetaxel chemotherapy for node-positive breast cancer patients: the FNCLCC PACS 01 Trial. Journal of Clinical Oncology 2006; 24:5664-71.

Sledge GW, Neuberg D, Bernardo P, Ingle JN, Martino S, Rowinsky EK, Wood WC. Phase III trial of doxorubicin, paclitaxel and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: An intergroup trial (E1193). Journal of Clinical Oncology 2003;21:588-592.

Sparano JA, Wang M, Martino S, Jones V, Perez EA, Saphner T, Wolff AC, Sledge GW, Jr., Wood WC, Davidson NE. Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med 2008; 358:1663-71.

Valero V, Holmes FA, Walters RS, Theriault RL, Esparza L, Fraschini G, Fonseca GA, Bellet RE, Buzdar AU, Hortobagyi GN. Phase II trial of docetaxel: a new, highly effective antineoplastic agent in the management of patients with anthracycline-resistant metastatic breast cancer. Journal of Clinical Oncology 1995; 13:2886-94.

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumour agents VI. The isolation and structure of taxol, a novel antileukemic and antitumour agent from *Taxus brevifolia*. J Am Chem Soc 1971;93:2325-2327.

Sequential Epirubicin-Docetaxel-CMF as adjuvant therapy of node-positive early stage breast cancer: the Taxit 216 randomized trial

The Taxit 216 Investigators
(including Forestieri V.)

IN PREPARATION

ABSTRACT

Background: Docetaxel is among the most active drugs for breast cancer. This trial was aimed at comparing the efficacy and tolerability of a block-sequential chemotherapy regimen containing docetaxel to a standard anthracycline-based regimen as adjuvant therapy in node-positive (N+) early breast cancer.

Methods: Between July 1998 and July 2002, 972 N+ early breast cancer patients were randomized to either arm A (E→CMF): Epirubicin (E) 120 mg/m2 iv d1 q21 x 4 cycles followed by Cyclophosphamide 600 mg/m2 iv, Methotrexate 40 mg/m2 iv and Fluorouracil 600 mg/m2 iv (CMF) dd1,8 q28 x 4 cycles or arm B (E→T→CMF) in which Docetaxel 100 mg/ m2 iv (T) d1 q21 x 4 cycles was administered after the 4th cycle of E and before the 1st cycle of CMF. Treatment allocation was performed by a computer program using a minimization algorithm. Stratification factors were: center, lymph node involvement (1 to 3, 4 to 9, > 10), estrogen receptor status (negative/positive/unknown), menopausal status (pre/post). The study was designed to detect a hazard ratio of 0.70, assuming an α of 0.05 (two-sided), a power of 0.80 and an expected DFS in Arm A of 0.65 at 5 years. This required 480 pts per Arm and 250 events. Final results are reported according to the standardized system for efficacy end-points (STEEP system).

Results: As of November 30th 2007, 486 pts were enrolled in arm A and 486 in arm B, 278 primary events were recorded and the median follow up was 62 months. Invasive disease-free survival (IDFS) at 5 years was 74% in arm B *vs* 68% in arm A (p=0.13) with an estimated adjusted Hazard Ratio (HR) of 0.82 (95%CI: 0.64-1.03). Recurrence-free survival (RFS), whose estimate does not take into account second primaries, was significantly better for arm B than for the arm A (76% *vs* 69%, p = 0.0332 with a HR of 0.75 (0.59-0.96). A statistically significant improvement was also observed for overall survival (OS). With a total of 142 deaths recorded, estimated OS at five years was equal to 90% for arm B and 85% for arm A (p=0.0168; HR=0.67, 95%CI: 0.48-0.94).

Conclusions: The block-sequential E→T→CMF regimen yields a significant improvement of RFS and OS as compared to E→CMF.

INTRODUCTION

Adjuvant chemotherapy is able to reduce the risk of recurrence and death of radically-resected early breast cancer (EBC) and anthracycline-based combinations have been shown on average to be superior to older combinations, like CMF (cyclophosphamide-methotrexate-fluorouracil) (1). The Taxit 216 trial was designed by Italian investigators in 1998 in the attempt to improve the efficacy of a standard adjuvant chemotherapy for node-positive early breast cancer. At that time, the pivotal role of anthracycline-based chemotherapy had just been established by the overview analysis of all randomized trials conducted by the Early Breast Cancer Trialists' Collaborative Group (2). Compared with the combination of cyclophosphamide, methotrexate, and fluorouracil (CMF), anthracycline-based regimens appeared to reduce on average the annual breast cancer death rate by approximately 12%. These findings encouraged the diffusion of anthracycline-based regimens and, in the absence of direct comparison among various anthracycline-based schedules, different regimens were adopted as standard treatment worldwide based on local preference and attitude.

In this scenario, a sequential regimen consisting of 4 courses of doxorubicin followed by various courses of CMF gained widespread acceptance in Europe. This was fuelled by the results of a randomized trial by Bonadonna et al. (3, 4) in which this block-sequential regimen compared favorably with a regimen alternating doxorubicin and CMF courses. This observation was also consistent with mathematical models that predicted better outcomes with block-sequential therapy than with an alternating regimen of non–cross-resistant agents (5). Therefore, despite the lack of trials directly comparing the block-sequential therapy with the classical CMF regimen, this regimen was regarded as a standard treatment by many clinicians in Europe and was, thus, chosen as standard reference arm by the Taxit 216 investigators. Nonetheless, the superiority of the block-sequential regimen over classical CMF has been more recently demonstrated for both anthracycline compounds, doxorubicin (6) and epirubicin (7), thus providing further support to the reference arm in our trial.

Taxanes were introduced for treatment of advanced breast cancer in the 1990s and their potential utility as adjuvant therapy was well acknowledged in 1998. Docetaxel, in particular appeared to be a highly effective compound as monochemotherapy in the treatment of metastatic breast cancer and its activity in anthracycline-resistant disease was well defined. Phase II studies showed a response rate ranging from 48.2% in anthracycline resistant patients to 38.8% in anthracycline refractory patients (8, 9). These data were confirmed by the phase III study conducted in anthracycline failure patients in which docetaxel was compared to the combination Mitomycin C vinblastine (10).

In the TAXIT 216 trial we aimed to assess the efficacy of adding docetaxel in a block-sequential fashion to a regimen with doxorubicin followed by CMF. In accordance with the Norton-Simon model (5), this regimen should warrant the highest dose-intensity for each drug used at standard dose while theoretically limiting the increase of toxicity, thus possibly yielding the best chance of tumor eradication.

METHODS

Study Population

Women first eligible for the study were between 18 and 64 years old and had undergone primary surgery with clear margins (ie, modified mastectomy or tumorectomy) plus axillary dissection for unilateral operable carcinoma of the breast (stage II-IIIa). Randomization was required within 6 weeks since breast cancer surgery.

In the original protocol, women were eligible if they had histologically proven tumor involvement in at least 4 axillary lymph-nodes (out of a minimum of 10 nodes removed). The trial was amended one year later to allow inclusion of patients with 1-3 axillary metastases and until 70 years old. Other main eligibility criteria included: baseline left ventricular ejection fraction (LVEF), as measured by echocardiography or MUGA scan, above the lower normal limit of each participating institution; adequate hematologic (granulocyte count $\geq 2x10^9$/L, platelet count $\geq 100x10^9$/L), hepatic (transaminases $\leq$ 1.5 x the upper limit of normal [ULN], alkaline phosphatases $\leq$ 2.5 x ULN, and bilirubin $\leq$ ULN) and renal (serum creatinine $\leq$ 140 µmol/L [1.6 mg/DL] or creatinine clearance $\geq$ 60 ml/min) function. Major exclusion criteria included pregnancy, documented history of cardiac disease contraindicating anthracyclines, previous cancer (except treated basal cell and squamous cell carcinoma of the skin or cancer of the uterine cervix), previous radiation therapy, hormone therapy, or chemotherapy for breast cancer, peripheral neuropathy > grade 2 according to the NCI Common Toxicity Criteria v2.0. Potentially eligible patients underwent staging by bone scan, chest x-ray, abdominal ultrasound, and contralateral mammography. Estrogen receptor (ER) status (positive vs negative) was evaluated by immunohistochemistry with a cut-off of 10% of cells with specific staining. Written informed consent was obtained before randomization. The protocol was reviewed and approved by the ethics committee/institutional review board and the study was conducted according to the Declaration of Helsinki and European Good Clinical Practice requirements.

Randomization procedure and Treatments

This study was an open-label, multicenter, randomized trial. Patients were randomized to the treatment groups through an automated minimization procedure that used center, lymph node metastases (1-3, 4-9, 10+), ER status (negative, positive, unknown) and menopausal status (pre/post) as stratification factors. Randomization was done centrally by fax at the coordinating center (University Federico II, Napoli, Italy).

Patients were assigned in a 1:1 ratio to receive epirubicin 120 mg/m² on day 1 every 21 days for 4 cycles followed by CMF 600/40/600 mg/m² on day 1,8 every 28 days for 4 cycles (arm A: E→CMF) or the same treatment with the addition of docetaxel 100 mg/m² on day 1 every 21 days for 4 cycles in between the 4 cycles of epirubicin and the 4 cycles of CMF (arm B: E→D→CMF). Therefore, patients in arm A were assigned to receive a total of 8 cycles of chemotherapy, while patients in arm B were assigned to receive a total of 12 cycles of chemotherapy. A third arm was initially open only for N>4+ patients at some selected centers to test feasibility and efficacy of a dose-intensified regimen, which included 4 cycles of dose-dense epirubicin (120mg/m² d1 q14) followed by 4 cycles of dose-dense docetaxel (100mg/m² d1 q14) and then 3 cycles of high-dose cyclophosphamide (3000 mg/m² d1 q21) (arm C: E_{dd}→D_{dd}→C_{hd}). A feasibility analysis was planned after inclusion of the first 25 patients on arm C using pre-specified safety criteria. This analysis led to early closure of this dose-intensified arm. Irrespective of arm, chemotherapy was delivered on an outpatient basis: docetaxel was infused over 1-hour period with routine steroid premedication, to prevent docetaxel-related hypersensitivity or fluid retention, starting 12 hours before and ending 18 hours after the docetaxel infusion. Antiemetics (5-HT3 receptor antagonists) were prescribed routinely before each cycle. Primary prophylaxis with granulocyte colony-stimulating factors (G-CSF), but not with antibiotics, was mandatory for arm C, while it was prohibited for arm A and arm B. In the event of an absolute neutrophil count (ANC) less than 1.5 x 10^9/L or a platelet count less than 100 x 10^9/L on day 1 of each cycle, treatment was delayed until recovery. Prophylactic G-CSF was recommended for subsequent cycles in case of a treatment delay due to neutropenia of more than 7 days or if the patient had suffered febrile neutropenia or grade 3-4 infection. If despite G-CSF treatment these problems persisted, a 25% dose-reduction was required for further chemotherapy administrations. The same dose-reduction was required in case of >7 days delay due to piastrinopenia and generally if a severe non-hematologic toxicity developed. If on day 8 of the CMF cycle, ANC was < 1.0 x 10^9/L and/or platelets were <100 x 10^9/L, chemotherapy was omitted and the subsequent cycle was started on day 21 instead of day 28.

Radiation therapy was mandatory after breast-conserving surgery and was delivered after completion of chemotherapy. No specific recommendations were given for post-mastectomy radiation therapy, which was delivered according to the guidelines of each center.

Tamoxifen therapy at 20mg/day for 5 years therapy was recommended after completion of chemotherapy to pre-menopausal patients with ER-positive tumors and to all post-menopausal patients irrespective of ER status, according to current practice at the time the protocol was developed.

Statistical Considerations

End-Points

As planned in the protocol, primary endpoint was Disease Free Survival (DFS) defined as the time between the date of randomization and the date of local or distant recurrence or contralateral breast cancer or second primary malignancy or death from any cause, whichever occurred first. No specification was given in the protocol as to whether consider ductal carcinoma in situ (DCIS), either contralateral or ipsilateral, as an event.

Quite recently, standardized definitions for efficacy endpoints (STEEP system) in adjuvant breast cancer trials have been proposed by a multidisciplinary panel of experts with the aim of reducing inconsistencies of results across clinical trials (11). Thus we decided to report the results according to such a system. Primary end-point was accordingly re-defined Invasive-DFS (IDFS), which excluded DCIS from the events of interest (11).

Efficacy results were also provided for Overall Survival (OS), and Recurrence-free survival (RFS) (11). OS was defined as the time from randomization to death from any cause. RFS was defined as the time between the date of randomization and the date of local or distant recurrence or death from any cause, whichever occurred first; thus contralateral breast cancer or second primary (non-breast) cancers were excluded (11).

Sample size calculation

The trial was originally designed to enrol 752 patients, 732 for the comparison of arm A *vs* arm B plus 120 patients for the comparison of arm B *vs* arm C. This was based on an expected 5yr DFS equal to 0.55 in arm A (3), a 10% absolute improvement in arm B and a further 15% absolute gain in arm C, with a type I error of 0.05 (two sided) and a power of 80%. In June 1999 inclusion criteria were amended, allowing the inclusion of subjects with 1 to 3 axillary metastases, and sample size was re-estimated accordingly, leading to a total sample size of 914 patients (794 for the comparison of arm A *vs* arm B plus 120 patients for the comparison of arm B *vs* arm C). Upon closure of arm C for toxicity the Steering Committee decided to re-evaluate again the sample size of the study to possibly increase the power of the first comparison. For this final calculation, expected 5yr DFS in arm A was set to 0.65 based on the results, which had become available meanwhile, of a previous trial evaluating the efficacy of a block-sequential anthracycline->CMF regimen in node-positive patients (6). Assuming an absolute improvement for the experimental arm (arm B) of 9% (HR equal to 0.70), a type I error (α) equal to 0.05 (two sided) and a power of 80%, it was estimated that a

total of 960 subjects and 250 events would be needed for the final analysis. All sample size adjustments were done blinded to data.

Statistical analysis

All efficacy analysis were done on an intention-to-treat basis. All subjects receiving at least one treatment dose were considered evaluable for efficacy analysis. Time-to-event curves were estimated with Kaplan-Meier (K-M) product limit, and statistical significance was assessed with a 2-sided log-rank test. Adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated using a Cox proportional hazards model that included treatment, lymph node metastases (1-3, 4-9, 10+), ER status (negative, positive, unknown) and menopausal status (pre/post) as covariates.

Exploratory subgroup analysis were reported as 'Forest plot', with 95% confidence intervals, focusing on possible interactions with treatment rather than strictly relying on statistical significance.

All patients who received treatment were considered for toxicity analysis. Up to six laboratory exams were planned for each cycle. Statistical analysis of toxicity was done in two ways. First, an exact linear permutation test was applied to allow for the ordinal nature of toxicity grades (Cytel 7 software). Second, an exact chi-square test was applied comparing severe (grades 3 to 4) versus non-severe (grades 0 to 2) toxicity.

Compliance to treatment was reported both on a per patient and a per cycle basis, according to treatment actually received. This analysis was descriptive only.

All the analysis were performed with SAS version 8.2 (SAS Inc., Cary, NC, USA) and graphs were made with R 2.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients characteristics

From July 1998 to July 2002, 998 patients were randomized in to the trial (arm A: n = 486; arm B: n = 486; arm C: n = 26).

After inclusion of 26 patients in arm C, a planned interim safety analysis was conducted to evaluate the feasibility of this dose-intensified treatment according to the planned feasibility rules. At this analysis, all rules were met except for the development of a grade 4 skin toxicity in one patient, which led to early closure of the arm. Therefore, no further results are reported for the arm C.

Baseline characteristics of patients in the arms A and B are reported in table 1. Baseline characteristics of all randomized patients were well balanced across the two treatment groups. Median age of patients was 51 years (range 23-74). The study included an equal number of pre- and post-menopausal women. Two thirds of patients were ER-positive.

Adherence to Treatment

The planned number of chemotherapy cycles was delivered to 91% patients in arm A and 74% in arm B. Reasons for treatment discontinuation are reported in table 2. Once separate drugs are considered, the planned number of four cycles was achieved in 96%, 83% and 93% of patients for epirubicine, docetaxel and CMF, respectively, without difference between arms.

Chemotherapy administration was delayed in 18% of cycles for arm A and 20% of cycles in arm B. A dose reduction was applied in about 4% of cycles in arm A *vs* 7% in arm B. With specific regard to docetaxel administration, a delay occurred in 21% of cycles and the dose was reduced in approximately 11% of cycles.

Efficacy

At the end date of November 30th 2006, 142 patients had died and median follow up of alive patients was equal to 62 months. According to the primary endpoint (IDFS) 278 events had occurred and their distribution between treatment arms is reported in table 3.

The experimental arm improved IDFS, although the difference did not reach statistical significance (log-rank p-value = 0.134). The estimated probability of

being free of any IDFS event at 5 years was equal to 0.74 for experimental arm and 0.68 for control arm. A statistically significant improvement was conversely observed for OS. Estimated probability of being alive at five years was equal to 0.90 for experimental arm and 0.85 for control arm (log-rank p-value = 0.0168). RFS curves, whose estimate do not take into account breast and non-breast second primaries. Estimated probability of being recurrence-free at five years was significantly better for the experimental arm than for the control arm (0.76 *vs* 0.69, respectively: log-rank p-value = 0.0332).

Multivariable analysis that included treatment, lymph node metastases, ER status and menopausal status as covariates confirmed these results. Hazard ratios and 95% confidence intervals for the three efficacy endpoints are reported in table 4. An exploratory analysis was carried out according to patient age, menopausal status, ER status and lymph node status to check whether these factors could modify the relative efficacy of the experimental arm as compared to the control arm. There was no evidence of heterogeneity of treatment efficacy in the various subgroups of patients.

Toxicity

Febrile neutropenia was significantly more frequent for patients in the experimental arm (11.6% *vs* 6.2%). Observed grade 3-4 neutropenia rates were much higher than commonly reported in both arms (84% *vs* 90% of subjects in arm A and arm B, respectively) and differed significantly (p=0.009). This very high incidence of neutropenia may possibly be due to the many laboratory exams (up to six) that were requested by the protocol for each chemotherapy cycle, thus yielding a high chance of registering the neutropenic nadir. Indeed, when the analysis was limited to laboratory values registered approximately at the time planned for recycling (day 21 for epirubicine and docetaxel; day 28 for CMF) grade 3-4 neutropenia rates dropped down to 10% *vs* 12% for arm A *vs* arm B, respectively and the difference was no more statistically significant (Fisher's exact test: p=0.42). Severe anemia and thrombocytopenia were uncommon.

More patients in arm B experienced at least one severe adverse event. Severe allergic reactions were rare (1.6% of patients) but they occurred only in the experimental arm. Peripheral edema was more frequent in the docetaxel arm but it was severe only in 3 patients (0.6%). Neurotoxicity was also more frequently reported for patients in arm B, but was only occasionally of grade 3 (1%). Other severe toxicities that were more frequent among docetaxel patients included: asthenia, arthralgy, myalgia, diarrhea, stomatitis, skin and nail disorders.

Overall, 5 non-breast cancers were recorded in arm A, including 1 small cell lung cancer, 1 thyroid cancer, 1 pancreatic carcinoma, 1 ovary carcinoma and 1

unspecified uterine cancer. Twelve non-breast cancers were observed in arm B: 2 colon cancers, 2 thyroid cancers, 1 melanoma, 1 kidney carcinoma, 1 tonsil cancer, 1 PNET, 1 ovarian cancer, 1 endometrial cancer, 1 cancer of the uterine cervix and 1 unknown cancer. No acute myeloid leukaemia or myelodisplastic syndrome have been recorded to date.

DISCUSSION

The Taxit 216 trial demonstrates that the introduction of four cycles of docetaxel into a block-sequential epirubicin-CMF regimen reduces the risk both of recurrence and of death of node-positive EBC patients. The effect on the risk of recurrence does not reach statistical significance when the analysis concerns the primary end-point of the study, i.e the IDFS. However, it emerges as statistically significant when the analysis focuses on the RFS. This apparent discrepancy may be explained by the inclusion of non-breast second primary tumors among the event of interest for the estimation of IDFS according to the STEEP system (11).

It is well acknowledged, indeed, that the inclusion of these non-breast cancer events for the estimation of the end-point avoids problems that arises from misdiagnosing a distant recurrence as a second primary cancer, but it has the drawback of diluting any treatment effect, should these second primaries not be related to the therapy under study (11).

In our trial, a small and non-statistically-significant, excess of non-breast second primary tumors is reported for the experimental arm. However, if we look at the incidence of second cancers across all randomized trials assessing a taxane-based adjuvant regimen reported in extenso so far, we note that there is overall no evidence of excess of non-breast cancer events for the taxane-based arms.

Furthermore, it appears that the incidence of non-breast primaries reported in our experimental arm is around the average of the other trials, while a very low incidence is registered for the control arm in our trial. These figures suggest that the imbalance of non-breast cancer events in the Taxit 216 trial is very probably unrelated to the treatment and occurred by chance. Therefore, their inclusion into the primary end-point may have weakened the discriminatory power of the analysis. In this situation, a better estimate of the therapeutic effect may come from the RFS analysis, which appeared, in our trial, significantly improved by the addition of docetaxel (about 25% relative risk reduction). Consistently with this, the experimental arm also showed a statistically significant advantage in OS (about 33% relative risk reduction).

Several phase III randomized trials have attempted to evaluate the effect of taxanes, combined with or in sequence with anthracycline-based regimens, in the adjuvant treatment of EBC patients. Most, but not all, the efficacy data so far available show a significant reduction of the risk of recurrence for the taxane-based treatment versus the control anthracycline-based treatment (12-24). On the other hand, a benefit in OS has been found only in a few trials (13, 14, 16, 20), casting doubts about the worth of such drugs in the adjuvant setting. However, a recent meta-analysis of all randomized trials available suggested that on average the addition of a taxane to an anthracycline-based treatment yields significant

benefits also in terms of OS (25). The final analysis of our trial is consistent with this finding lending further support to the use of such drugs, particularly docetaxel, in the adjuvant setting.

Controversy exists as to whether the anthracycline-based control arms in such trials could be considered standard control arms. Indeed, not all anthracycline-based regimens used as control arm in such trials have clearly proven superiority versus CMF, thus confounding the interpretation of the results. In such a situation, clinical preference should be given to those taxane-based regimens that have been compared to an adequate anthracycline-based control regimen.

The block-sequential E-CMF regimen used as control arm in the Taxit 216 trial is among the few control regimens in adjuvant taxane trials, if not the only one, that have succeeded on CMF in direct randomized comparisons (6, 7), making its results very solid. A similar block-sequential regimen, but with a lower number of cycles, has been used as control arm in the recently published BIG2-98 trial (23), leading to comparable results.

A crucial issue is whether taxanes should be combined with anthracyclines or whether they should be administered sequentially after an anthracycline-based regimen. Both options have theoretical advantages and drawbacks: combination regimens require dose-reduction for both compounds but may, in theory, exploit drug synergism; in sequential regimens, on the other hand, both compounds can be administered at optimal doses. Three decades ago, Norton and Simon reported that the growth of solid neoplasms could be described by Gompertzian curves, in which the rate of regrowth of a tumor increases as the tumor shrinks in response to therapy. The Norton – Simon hypothesis (5) predicted that this resistance might be overcome by switching from initial chemotherapy agents to new agents at the maximally tolerated dose.

The results of the Taxit 216 trial are consistent with this hypothesis. Other trials, however, have found similar results with regimens in which the taxane was given in combination with the anthracycline (14, 18). De Laurentiis et al, in their metanalysis of randomized trials (25), carried out an exploratory indirect comparison between block-sequential and combination regimens and did not find a statistically significant difference between the therapeutic effect of such regimens. However, because of the indirect nature of this comparison, it cannot be definitely excluded that there could be moderate but worthwhile differences in efficacy between these types of regimens, which can only be identified in a direct randomized comparison.

To date, the only trial reporting a direct randomized comparison between a sequential and combination taxane-based regimen is the BIG2-98 trial (23), showing an advantage for the sequential regimen of borderline statistically significance. However, design issues (ie, the longer treatment duration of the experimental arm, the different docetaxel/anthracycline doses, the unplanned

nature of this comparison) preclude definitive conclusions about this issue. Results of other adjuvant trials, such as BCIRG 005 and NSABP B-30 trials, that are testing sequential and concurrent anthracycline-docetaxel regimens, are eagerly awaited.

There is some controversy about whether taxanes produce consistent benefit across specific subgroups of patients. Single trial figures suggest that the benefits of taxanes may be lower, if not negligible, for N>4+ (14, 20) and for ER+ patients (12, 13, 16, 24). Other trials suggest that benefit differs between younger and older patients (14, 20, 22).

In our trial, docetaxel appears to reduce the risk of recurrence irrespective of ER status (ER+ *vs* ER-), nodal status (N1-3 *vs* N4-9 *vs* N10+), age (<=50 *vs* >50) and menopausal status (pre-menopausal *vs* post-menopausal). Although a fluctuation of the relative benefit is evident across some subgroups of patients, there is indeed no statistically significant heterogeneity among such estimates, thus indicating that this fluctuation only arises by chance. Therefore, our study indicates that neither the number of axillary lymph node metastases, or the age, or the menopausal status and ER status should be used in clinical practice to identify patients who may (or may not) benefit from our experimental regimen. Again, this is consistent with what reported on average by the above mentioned metanalysis of randomized trial (25).

Two limitations of our trials deserve discussion. First, in the context of the other relevant trials, the Taxit 216 trial is penalized by a smaller than average sample size. It may be argued that this decreases the value of the trial results. However, underpowered trials have by definition a high chance of not detecting a statistically significant difference between treatments (i.e, high chance of being false-negative trials). Conversely, once a statistically significant difference is detected, as in the case of our trial, the small sample size does not decrease the absolute strength of such an evidence, although it affects the precision of the point estimate for the HR. Second, our experimental treatment is longer than other taxane-based regimens and this may raise concerns as to whether it could cause excessive toxicity and patients' discomfort.

In this regard, our toxicity analysis appears reassuring. Hematologic severe adverse events were rare in our experimental arm except for G3-4 neutropenia. However, if we limit the analysis to the day planned for chemotherapy administration, severe neutropenia was not more frequent than in the control arm and it was inferior to what generally reported with other shorter taxane-based regimens (14, 20, 22). Febrile neutropenia affected more patients in the experimental arm than in the control arm (11.6% *vs* 6.2%), but its frequency was similar to what reported for shorter block-sequential regimens (20, 22) and much less than the 25% reported for the TAC regimen (14). Among other taxane-specific adverse events, severe neurotoxicity was rare (1%) and its frequency was

inferior to what reported for shorter paclitaxel-based regimens (13, 15, 22, 26, 27). Furthermore, if patients' preferences are concerned, recent data indicate that the duration of an adjuvant regimen is less important than expected for patients and that most of them are willing to accept a longer treatments if this is associated to an even marginal additional benefit (28). In this regard, as a balance to the longer duration, our regimen is the only one to yield so far an OS benefit in comparison to an adequate anthracycline regimen.

In conclusion, incorporating docetaxel into a block-sequential E-CMF regimen yields a significant reduction of the risk of recurrence and death for node-positive EBC. This effect is independent of the age and of the menopausal status of the patients so as of the ER status of the tumor and the number of axillary lymph node metastases. This advantage comes at the cost of an increased, but acceptable, toxicity and discomfort.

Table 1: Patient characteristics by treatment arm

		E → CMF (486)	E → T →CMF (486)
Age	<50	227(46.7)	213 (43.8)
	≥50	259 (53.3)	273 (56.2)
Menopause	Pre	244 (50.2)	243 (50.0)
	Post	242 (49.8)	243 (50.0)
T	1	194 (39.9)	219 (45.1)
	2	242 (49.8)	203 (41.8)
	3-4	50 (10.3)	64 (13.2)
Nodes	1-3	179 (36.8)	178 (36.6)
	4-9	193 (39.7)	198 (40.7)
	>=10	114 (23.5)	110 (22.6)
Histology	Ductal	390 (80.3)	378 (77.8)
	Lobular	59 (12.1)	71 (14.6)
	Other	37 (7.6)	37 (7.6)
ER	Negative	114 (23.5)	117 (24.1)
	Positive	319 (65.6)	315 (64.8)
	Unknown	53 (10.9)	54 (11.1)

Table 2: Reasons for discontinuation of chemotherapy by received treatment

Causes of interruption	E →CMF	E → T →CMF
Breast cancer relapse	3	7
Second primary malignancy	0	1
Adverse experience	17	31
Consent withdrawal	12	43
Death	0	1
Protocol deviation	0	2
Lost	0	3
Other	4	25
Not Reported	10	14
Total	**46**	**127**

Table 3: Patients with first IDFS events

	E →CMF (486)	E → T →CMF (486)
Breast cancer relapse	139 (28.6)	108 (22.2)
Loco\regional	25 (5.1)	19 (3.9)
Distant	114 (23.5)	89 (18.3)
Death	5 (1.0)	7 (1.4)
Second primary cancer	5 (1.0)	14 (2.9)
Breast	1 (0.2)	3 (0.6)
Other	4 (0.8)	11 (2.3)
Total IDFS events	149 (30.7)	129 (26.5)
None (event-free patients)	337 (69.3)	357 (73.5)

Table 4: Adjusted Hazard Ratios for different endpoints*

	Events		HR (95%CI)
	E →CMF	E → T →CMF	
IDFS	149	129	0.82 (0.64-1.03)
RFS	146	117	0.75 (0.59-0.96)
OS	85	57	0.67 (0.48-0.94)

*Adjusted for lymph node metastases, ER status and menopausal status.

REFERENCES

1. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687-1717, 2005

2. Polychemotherapy for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 352:930-942, 1998

3. Bonadonna G, Zambetti M, Valagussa P: Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. JAMA 273:542-547, 1995

4. Buzzoni R, Bonadonna G, Valagussa P, et al.: Adjuvant chemotherapy with doxorubicin plus cyclophosphamide, methotrexate, and fluorouracil in the treatment of resectable breast cancer with more than three positive axillary nodes. J Clin Oncol 9:2134-2140, 1991

5. Norton L, Simon R: The Norton-Simon hypothesis revisited. Cancer Treat Rep 70:163-169, 1986

6. De Placido S, De Laurentiis M, De Lena M, et al.: A randomised factorial trial of sequential doxorubicin and CMF vs CMF and chemotherapy alone vs chemotherapy followed by goserelin plus tamoxifen as adjuvant treatment of node-positive breast cancer. Br J Cancer 92:467-474, 2005

7. Poole CJ, Earl HM, Hiller L, et al.: Epirubicin and cyclophosphamide, methotrexate, and fluorouracil as adjuvant therapy for early breast cancer. N Engl J Med 355:1851-1862, 2006

8. Valero V, Holmes FA, Walters RS, et al.: Phase II trial of docetaxel: a new, highly effective antineoplastic agent in the management of patients with anthracycline-resistant metastatic breast cancer. J Clin Oncol 13:2886-2894, 1995

9. Ravdin PM, Burris HA, III, Cook G, et al.: Phase II trial of docetaxel in advanced anthracycline-resistant or anthracenedione-resistant breast cancer. J Clin Oncol 13:2879-2885, 1995

10. Nabholtz JM, Senn HJ, Bezwoda WR, et al.: Prospective randomized trial of docetaxel versus mitomycin plus vinblastine in patients with metastatic

breast cancer progressing despite previous anthracycline-containing chemotherapy. 304 Study Group. J Clin Oncol 17:1413-1424, 1999

11. Hudis CA, Barlow WE, Costantino JP, et al.: Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. J Clin Oncol 25:2127-2132, 2007

12. Buzdar AU, Singletary SE, Valero V, et al.: Evaluation of paclitaxel in adjuvant chemotherapy for patients with operable breast cancer: preliminary data of a prospective randomized trial. Clin Cancer Res 8:1073-1079, 2002

13. Henderson IC, Berry DA, Demetri GD, et al.: Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 21:976-983, 2003

14. Martin M, Pienkowski T, Mackey J, et al.: Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 352:2302-2313, 2005

15. Mamounas EP, Bryant J, Lembersky B, et al.: Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from NSABP B-28. J Clin Oncol 23:3686-3696, 2005

16. Fountzilas G, Skarlos D, Dafni U, et al.: Postoperative dose-dense sequential chemotherapy with epirubicin, followed by CMF with or without paclitaxel, in patients with high-risk operable breast cancer: a randomized phase III study conducted by the Hellenic Cooperative Oncology Group. Ann Oncol 16:1762-1771, 2005

17. Evans TR, Yellowlees A, Foster E, et al.: Phase III randomized trial of doxorubicin and docetaxel versus doxorubicin and cyclophosphamide as primary medical therapy in women with breast cancer: an anglo-celtic cooperative oncology group study. J Clin Oncol 23:2988-2995, 2005

18. Gianni L, Baselga J, Eiermann W: European Cooperative Trial in Operable Breast Cancer (ECTO): Improved freedom from progression (FFP) from adding paclitaxel (T) to doxorubicin (A) followed by cyclophosphamide methotrexate and fluorouracil (CMF). (Abs#513). J Clin Oncol 2005 ASCO Annual Meeting Proceedings. Vol 23, No. 16S:513, 2005

19. Jones SE, Savin MA, Holmes FA, et al.: Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide

as adjuvant therapy for operable breast cancer. J Clin Oncol 24:5381-5387, 2006

20. Roche H, Fumoleau P, Spielmann M, et al.: Sequential adjuvant epirubicin-based and docetaxel chemotherapy for node-positive breast cancer patients: the FNCLCC PACS 01 Trial. J Clin Oncol 24:5664-5671, 2006

21. Bear HD, Anderson S, Smith RE, et al.: Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer:National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 24:2019-2027, 2006

22. Martin M, Rodriguez-Lescure A, Ruiz A, et al.: Randomized phase 3 trial of fluorouracil, epirubicin, and cyclophosphamide alone or followed by Paclitaxel for early breast cancer. J Natl Cancer Inst 100:805-814, 2008

23. Francis P, Crown J, Di Leo A, et al.: Adjuvant chemotherapy with sequential or concurrent anthracycline and docetaxel: Breast International Group 02-98 randomized trial. J Natl Cancer Inst 100:121-133, 2008

24. Goldstein LJ, O'Neill A, Sparano JA, et al.: Concurrent Doxorubicin Plus Docetaxel Is Not More Effective Than Concurrent Doxorubicin Plus Cyclophosphamide in Operable Breast Cancer With 0 to 3 Positive Axillary Nodes: North American Breast Cancer Intergroup Trial E 2197. J Clin Oncol, 2008

25. De Laurentiis M, Cancello G, D'Agostino D, et al.: Taxane-based combinations as adjuvant chemotherapy of early breast cancer: a meta-analysis of randomized trials. J Clin Oncol 26:44-53, 2008

26. Citron ML, Berry DA, Cirrincione C, et al.: Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. J Clin Oncol 21:1431-1439, 2003

27. Sparano JA, Wang M, Martino S, et al.: Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med 358:1663-1671, 2008

28. Duric VM, Butow PN, Sharpe L, et al.: Comparing patients' and their partners' preferences for adjuvant chemotherapy in early breast cancer. Patient Educ Couns 72:239-245, 2008

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ORIGINAL REPORT

Taxane-Based Combinations As Adjuvant Chemotherapy of Early Breast Cancer: A Meta-Analysis of Randomized Trials

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ABSTRACT

Purpose
We conducted a meta-analysis of randomized trials that evaluated the efficacy of incorporating taxanes into anthracycline-based regimens for early breast cancer (EBC). We aimed to determine whether this approach improves disease-free survival (DFS) and overall survival (OS) and whether benefits are maintained across relevant patient subgroups.

Methods
Studies were retrieved by searching the PubMed database and the proceedings of major conferences. We extracted hazard ratios (HR) and 95% CIs for DFS and OS from each trial and obtained pooled estimates using an inverse-variance model.

Results
Thirteen studies were included in the meta-analysis (N = 22,903 patients). The pooled HR estimate was 0.83 (95% CI, 0.79 to 0.87; $P < .00001$) for DFS and 0.85 (95% CI, 0.79 to 0.91; $P < .00001$) for OS. Risk reduction was not influenced by the type of taxane, by estrogen receptor (ER) expression, by the number of axillary metastases (N1 to 3 *v* N4+), or by the patient's age/menopausal status. Sensitivity analysis showed that taxanes given in combination with anthracyclines, unlike sequential administration, did not significantly improve OS. However, the test for interaction showed that HR did not differ between the two schedules ($P = .54$). Taxane administration resulted in an absolute 5-year risk reduction of 5% for DFS and 3% for OS.

Conclusion
The addition of a taxane to an anthracycline-based regimen improves the DFS and OS of high-risk EBC patients. The DFS benefit was independent of ER expression, degree of nodal involvement, type of taxane, age/menopausal status of patient, and administration schedule.

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INTRODUCTION

Adjuvant chemotherapy based on combinations of cytotoxic drugs reduces the risk of recurrence of radically resected early breast cancer (EBC). Anthracycline-based combinations are generally more effective than earlier combinations like cyclophosphamide-methotrexate-fluorouracil (CMF) and have become the standard adjuvant chemotherapy for most patients with breast cancer.[1,2]

Taxanes have recently emerged as the most active cytotoxic agents for breast cancer. In the metastatic setting, these compounds were active in anthracycline-resistant disease, and in phase III trials, single-agent taxanes were at least as active as, and sometimes more active than, single-agent anthracyclines.[3-5] Furthermore, the combination of anthracyclines and taxanes resulted in a better response rate and, in some cases, a longer time-to-progression than standard anthracycline-based regimens.[6] Consequently, taxane-anthracycline combinations are now widely used as standard first-line treatment for advanced breast cancer.

The results of the phase III trials prompted randomized trials designed to evaluate the effect of taxanes, combined with or in sequence with anthracycline-based regimens, in the adjuvant treatment of EBC patients. Most, but not all, of the efficacy data published to date show a significant improvement in disease-free survival (DFS) for the taxane-based treatment versus the control anthracycline-based treatment.[7-20] Nonetheless, the role of taxane-based chemotherapy as adjuvant treatment of EBC remains controversial. For instance, a benefit in overall survival (OS) has been found only in a few trials.[8,10,14,17] In addition, it remains unclear whether taxane-based regimens

yield worthwhile benefits in patients with four or more axillary metastases (N4+), because some trials have shown, at subgroup analysis, a lower or no benefit for such patients.[14,17] However, one of the major issues is whether endocrine-responsive tumors benefit from adjuvant taxane-based therapy. Most trials carried out so far did not find a significant benefit in this subgroup of patients[7,8,10,16,20]; hence, a panel of international breast cancer experts proscribed taxane-based therapy for endocrine-responsive breast cancers.[1] Nonetheless, this apparent lack of benefit in single trials may well be due to chance or to the low statistical power of subgroup analyses.

In this scenario, we carried out a meta-analysis of randomized trials to address questions about the efficacy of adjuvant taxane-based therapy, particularly in relevant subgroups of EBC patients.

METHODS

The review was conducted according to a predefined written protocol developed by M.D.L. who also coordinated a discussion among all authors to reach a consensus about each specific methodologic issue.

Study Identification

Studies were identified by a computerized search of the PubMed database (years 2000 to 2006) using the following text words: "breast cancer and (paclitaxel or docetaxel)." A computerized search of the abstracts and presentations reported at the Annual Meetings of the American Society of Clinical Oncology held between 2000 and 2006 or at the San Antonio Breast Cancer Symposium from 2000 to 2005 was run to identify relevant unpublished studies. Lastly, all review articles and all cross-referenced manuscripts from retrieved articles were screened for pertinent studies.

Selection Criteria

To be included in the meta-analysis, retrieved studies had to fulfill the following inclusion criteria: (1) early breast cancer; (2) adjuvant therapy; (3) randomized trial comparing a taxane-anthracycline-based regimen with an anthracycline-based regimen. Studies meeting these criteria were excluded from the analysis if the retrieved paper was an earlier report of data updated in a subsequent article, abstract, or presentation.

Outcomes for Analysis

The main outcomes analyzed were DFS and OS. DFS events included second primary breast cancers, local or distant recurrences of the original cancer, or death, unless otherwise specified (Table 1). The data of all trials were based on the intention-to-treat principle, so they compared all women allocated one treatment with all those allocated the other, irrespective of compliance. The effect of the treatment for each single study was expressed as a hazard ratio (HR) of the taxane-based arm over the standard anthracycline-based arm. Thus, an HR greater than one favors the standard arm whereas an HR less than 1 favors the taxane-based treatment. 95% CIs were calculated for each point estimate.

Data Extraction

The following information was extracted from each report: study design, regimen details, allocated patients, nodal status, median follow-up, HRs for the whole study populations and for major patient subgroups (if available), and year of reporting. Data were independently extracted from each report by A.G. and M.G., who were blinded to each other, using a standardized data recording form. After extraction, data were reviewed and compared by M.D.L. and G.C. Instances of disagreement between the two data extractors were resolved by consultation.

Author-reported HRs with 95% CIs were used when possible. If 95% CIs were not directly reported, they were estimated by the P value of the log-rank statistics.[21] For two studies,[10,16] reported HRs referred to the standard arm rather than the taxane-based arm (ie, HR $>$ 1 favoring taxanes) and were, therefore, recalculated by taking the exponential of negative ln(HR) to keep consistency with other trials. When HRs were not directly reported in the original study, they were estimated indirectly using either the reported number of events and the corresponding P value for the log-rank statistics, or by reading off survival curves as suggested by Parmar et al.[21] To reduce reading errors, original survival curves were digitalized and enlarged, and data extraction was based on reading off electronic coordinates for each point of interest.

Data Synthesis

A pooled estimate of the HRs was computed by a fixed-effect model according to the inverse-variance method.[22] We also used the DerSimonian and Laird[23] random-effect model. This gives a more appropriate estimate of the average treatment effect when trials are statistically heterogeneous, and it usually yields wider CIs, thereby resulting in a more conservative statistical claim. Homogeneity assumption was checked with Cochran's Q statistics.[24] To obtain a quantitative measure of the degree of inconsistency in the results of studies, we calculated a Higgins' I^2 index. This index describes the percentage of total cross-study variation that is due to heterogeneity rather than chance.[25] Potential publication bias was estimated with the Begg-Mazumdar test[26] and the Egger test.[27] For all the analyses, a forest plot was generated to display results.

We carried out a sensitivity analysis by recalculating the pooled HR estimate for different subsets of studies based on relevant clinical features. This analysis serves to determine whether the pooled estimates are stable or whether they depend on some features of the studies included in the meta-analysis. Consequently, it shows whether or not the overall result would be affected by a change in the meta-analysis selection criteria.

A subgroup analysis was performed by pooling estimates for similar subsets of patients across trials where available. An interaction test between treatment effect and subgroup factors was calculated according to Deeks et al.[28] To estimate the absolute gains in DFS and OS, we calculated meta-analytic survival curves as suggested by Parmar et al.[21] All statistical calculations were done by computer routines developed in-house.

RESULTS

Results of Literature Search

Fourteen studies were identified.[7-20] Of these, the US Oncology study by Jones et al[18] was excluded because it investigated the role of a taxane in substitution of (and not in addition to) an anthracycline. Therefore, 13 studies were used in the main pooled analysis (22,903 patients). Table 1 presents the studies identified and their main characteristics.

Overall Effect of Taxanes on Risk of Recurrence

HRs for DFS were available, either directly or indirectly, for all 13 studies accounting for 5,829 events. Single-study HRs ranged from 0.63 to 0.97 and were statistically significant in seven studies. Figure 1 reports the estimated pooled HR for all studies. The reduction of risk of recurrence was highly significant ($P < .00001$) in patients receiving taxane-based therapy, and there was no significant difference regarding the type of taxane administered (test for interaction, $P = .16$). In addition, there was no evidence of heterogeneity among trials ($P = .32$; $I^2 = 12.1\%$) or publication bias (Begg-Mazumdar test, $P = .14$; Egger test, $P = .08$). The sensitivity analysis (Table 2) shows that DFS was significantly improved even when the meta-analysis was restricted to trials of taxanes in combination regimens, to trials of taxanes in sequential regimens, or to studies of node-positive patients only.

Overall Effect of Taxanes on the Risk of Death

One study[7] did not report OS data, and thus the meta-analysis of the effect of taxanes on the risk of death is limited to 12 studies and 22,379 patients, accounting for 3,329 deaths. Single-study HRs ranged from 0.41 to 1.03 and were statistically significant in four studies. The estimated pooled HR for all the studies shows a highly significant

Table 1. Main Characteristics of the Studies Included in the Meta-Analysis

Trial	N Status	No. of Patients	Median FU2 (months)	Author-Reported Data								Notes
				DFS				OS				
				Rate	*P*	95% CI	Events	Rate	*P*	95% CI	Deaths	
M.D. Anderson (MDACC2002)[7] 2002*	N-/N+	524	60	0.70	.09	0.47 to 1.07		NR				Primary end point is RFS, but its definition is not reported
Design												
-F500 A50 C500 × 8		259					53					
-P250 × 4-> F500A50 C500 × 4		265					39					
CALGB 9344[8] 2003†	N+	3,121	69	0.83	.0013	0.73 to 0.94		0.82	.0061	0.71 to 0.95		DFS does not include second breast primaries
Design												
-A(diff.doses)C × 4		1,570					563				400	
A(diff.doses)C × 4-> P175 × 4		1,551					491				342	
Anglo-Celtic[15] 2005	N-/N+	363	32	NR				NR				Primary end point is RFS (relapse-free survival), but its definition is not reported
Design												
-A60 C600 × 6-> S		180					55				28	
-A60 D75 × 6-> S		183					45				25	
BCIRG 001[14] 2005	N+	1,491	55	0.72	.001	0.59 to 0.88		0.70	.008	0.53 to 0.91		DFS includes also second nonbreast primaries
Design												
-F500 A50 C500 × 6		746					227				130	
-D75 A50 C500 × 6		745					172				91	
ECOG E 2197[16] 2005	N-/N+	2,885	59	1.03	.70	0.86 to 1.25		1.09	.49	0.85 to 1.40		Data are from oral presentation. Author reported HRs are for taxane-based arm over control arm (HR> 1 favors taxanes)
Design												
-A60 C600 x 4		1,441					219				125	
-A60 D600 x 4		1,444					213				117	
ECTO[11] 2005‡	N-/N+	1,355	43	0.65	.01	0.47 to 0.90		0.71	.16	NR		Data are from oral presentation and pertain to Cox analysis. Primary end point is freedom from progression (FFP) defined as the interval from random assignment to first evidence of breast cancer progression or relapse
Design												
A-A60 x 4-> CMF × 4		453					91				41	Events and Deaths: Arm B *v* Arm A
B-A60 P200 x 4-> CMF × 4		451					63				30	Events and Deaths: Arm B *v* Arm A
C-A60 P200 × 4-> CMF × 4->S		451					78				32	Events and Deaths: Arm B *v* Arm A
GEICAM 9906[13] 2005	N+	1,248	46	0.63	.001	0.48 to 0.83		0.74	.14	NR		HR are from Cox analysis; DFS definition not reported
Design												
-F600 E90 C600 × 6		634					128				49	
-F600 E90 C600 x 4→ P100 × 8 wks		614					83				34	
HeCOG 10/97[10] 2005¶	N-/N+	595	62	1.16	.31	0.87 to 1.55		2.42	.02	1.17 to 4.99		HR are from Cox analysis; author-reported HRs are for taxane-based arm over control arm (HR > 1 favors taxanes)
Design												
-E110 × 4->°CMF × 4		298					98				61	
-E110 × 3->P250 × 3->°CMF × 3		297					91				53	
NSABP B28[12] 2005	N+	3,059	64.6	0.83	.006	0.72 to 0.95		0.93	.46	0.78 to 1.12		DFS includes second nonbreast primaries
Design												
-A6 0C600 × 4		1,531					463				255	
-A60 C600 × 4-> P225 × 4		1,528					400				243	

(continued on following page)

Table 1. Main Characteristics of the Studies Included in the Meta-Analysis (continued)

Trial	N Status	No. of Patients	Median FU2 (months)	Author-Reported Data: DFS Rate	DFS *P*	DFS 95% CI	DFS Events	OS Rate	OS *P*	OS 95% CI	OS Deaths	Notes
BIG 2-98[20] (TAX-315) 2006§	N+	2,887	62.5	0.86	.051	0.74 to 1.00		0.92	.34	0.75 to 1.13		Data are from oral presentation
				(Arm 3 + Arm 4 *v* Arm 1 + Arm 2)				(Arm 3 + Arm 4 *v* Arm 1 + Arm 2)				DFS definition not reported
Design												
1-A75 × 4-> CMF × 3		481					266				143	
2-A60 C600 × 4-> CMF × 3		487										
3-A75 × 3->D100 × 3->CMF × 3		960					466				260	
4-A50 D75 × 4-> CMF × 3		959										
NSABP B27[9] 2006	N-/N+	2,404	77.9	0.90	.24	0.76 to 1.06		1.08	.51	NR		DFS includes also all clinically inoperable, residual disease at surgery, and second nonbreast primaries; Arm III *v* Arm I
Design												
I-A60 C600 × 4-> S		802					276				157	
II-A60 C600 × 4->D100 × 4-> S		803					260				156	
III-A60 C600 × 4-> S->D100 × 4		799					254				171	
PACS 01[17] 2006	N+	1,999	60	0.82	.034	0.69 to 0.99		0.73	.014	0.56 to 0.94		Data are from oral presentation and pertain to Cox analysis; DFS definition not reported
Design												
-F500 E100 C500 × 6		1,003					264				130	
-F500 E100 C500 × 3-> D100 × 3		996					218				135	
TAXIT 216[19] 2006	N+	972	53.6	0.79	.058	0.61 to 1.00		0.72	.08	0.5 to 1.04		Data are from oral presentation; DFS includes second nonbreast primaries
Design												
-E120 × 3->CMF × 3		486					138				70	
-E120 × 3-> D100 × 3->CMF × 3		486					115				51	

NOTE. Both arms and all drugs q14.
Abbreviations: P, paclitaxel; D, docetaxel; E, epidoxorubicin; A, doxorubicin; S, surgery; NR, not reported, DFS, disease-free survival, OS, overall survival; RFS, relapse/recurrence-free survival; HR, hazard ratio.
*M.D.Anderson: fluorouracil: days 1,4; doxorubicin, 72 hours continuous infusion; paclitaxel, 24 hours continuous infusion.
†CALGB 9344: A (differing doses): adriamicin 60, 75, or 90 mg/m^2.
‡ECTO CMF: cyclophosphamide, 600 mg/m^2; methotrexate, 40 mg/m$^{2:}$ fluorouracil, 600 mg/m^2 days 1,8 q28.
§HeCOG 10/97 CMF: cyclophosphamide, 840 mg/m^2; methotrexate, 57 mg/m^2; fluorouracil, 840 mg/m^2.
‖TAXIT 216 CMF: cyclophosphamide: 600 mg/m^2; methotrexate, 40 mg/m^2; fluorouracil, 600 mg/m^2 days 1,8 q28.
¶BIG 2-98 CMF: cyclophosphamide 100 mg/m^2 PO days 1-14; methotrexate, 40 mg/m^2 fluorouracil, 600 mg/m^2 days 1,8 q28.

reduction of the risk of death for patients receiving a taxane-based therapy ($P < .0001$; Fig 2). There was no statistical heterogeneity among studies ($P = .10$; $I^2 = 36.2\%$) or evidence of publication bias (Begg-Mazumdar test, $P = .27$; Egger test, $P = .19$). The improvement in OS was similar for the paclitaxel group and for the docetaxel group (test for interaction, $P = .55$). The sensitivity analysis (Table 2) shows that OS was not significantly improved when the meta-analysis was restricted to studies of combination regimens, and there was significant heterogeneity among trials ($P = .02$). However, the test for interaction ($P = .54$) indicates that the risk reduction observed for this subset of studies was not significantly different from that observed for studies of sequential regimens.

Effect of Taxanes in Specific Subgroups of Patients

Because of lack of information in most trials, subgroup analysis according to estrogen receptor (ER) status (ER positive *v* ER negative), nodal status (N1 to 3 *v* N4+), age (≤ 50 *v* > 50), menopausal status (premenopausal *v* postmenopausal) and HER-2 status (HER-2 positive *v* HER-2 negative) was possible only for subsets of trials and only for DFS. Treatment effect according to ER status was available, either directly reported or indirectly derived, for 10 studies and a total of 17,324 patients. Pooled HR estimates indicate that taxanes significantly reduced the risk of recurrence for both ER-positive and ER-negative patients (Fig 3). There was indeed no statistically significant difference between the HRs in the two patient subgroups (test for interaction, $P = .31$). This was independent of whether paclitaxel or docetaxel was administered (data not shown). HR estimates by nodal status were available for four trials and 6,179 patients. The pooled HR for DFS was similar for patients with one to three positive lymph nodes and for patients with four or more positive lymph nodes (test for interaction, $P = .63$; Fig 4).

Treatment effect according to age/menopausal status is reported in Figure 5. Only three trials reported DFS information based on age grouping (≤ 50 *v* > 50 years), and two other trials reported data based on menopausal status. Because postmenopausal status usually arises

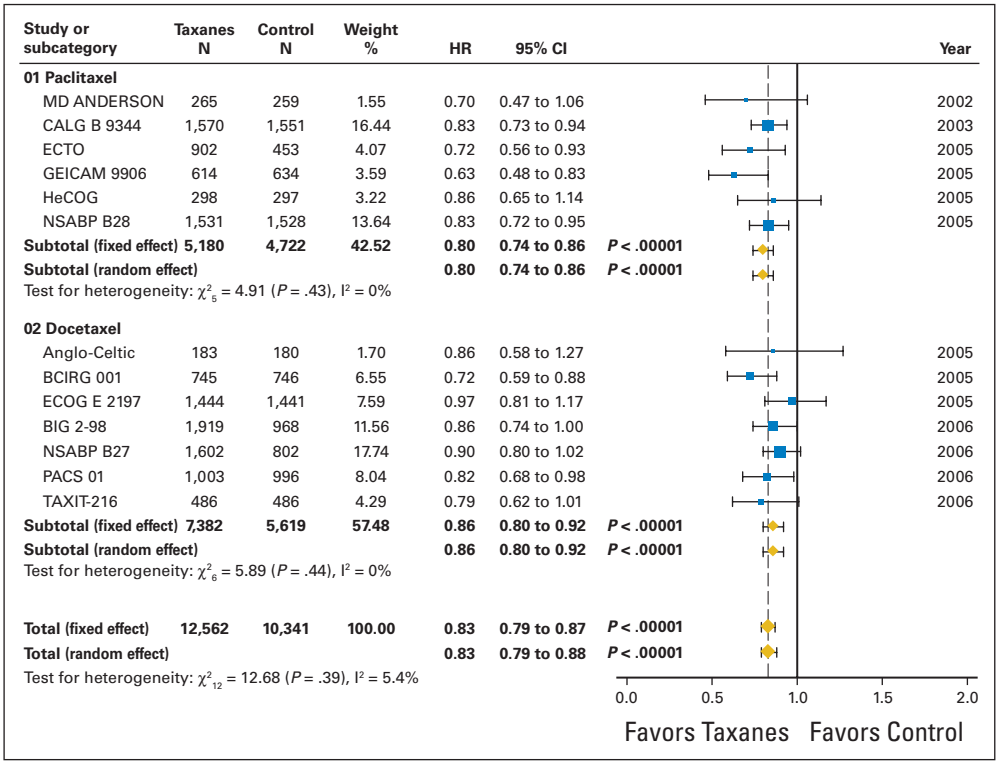

Fig 1. Meta-analysis of disease-free survival (DFS). Studies are grouped based on the type of taxane used. Within each group, trials are ordered by year of reporting and by alphabetical order; squares on the hazard ratio plot are proportional to the weight of each study; weighting is based on the inverse variance method.

around the age of 50 years, these two groupings are partially interchangeable, and we analyzed them together to obtain a more precise estimate. Taxanes resulted in a significant risk reduction for both age ≤ 50 years/premenopausal and older than 50 years/postmenopausal patients. The large benefit observed in the older group in the Spanish Breast Cancer Research Group (GEICAM) trial resulted in statistical heterogeneity. However, there is no statistically significant difference between the HRs for the two subgroups of patients (test for interaction, $P = .13$). Results were similar when the analysis was restricted to age grouping (data not shown). Only two studies reported DFS data according to HER-2 expression, and, therefore, HR estimates are less reliable (Fig 6). However, there was no interaction between HER-2 expression and taxane administration in terms of reduction of risk of recurrence (test for interaction, $P = .28$).

Absolute Benefits of Taxane Therapy

Figure 7 depicts metacurves of DFS and OS derived from stratified pooling of the data of the trials. Cumulative estimates of probability of DFS and OS are drawn up to 5 years. Estimates became unreliable beyond this time because follow-up is still immature. Based on these curves, the estimated absolute risk reduction at 5 years, gained by adding a taxane to an anthracycline-based adjuvant regimen, is approximately 5% for DFS and 3% for OS.

DISCUSSION

We identified 13 studies that assessed the addition of a taxane to an anthracycline-based regimen. These studies accounted for a total of 22,452 randomly assigned women, 5,829 recurrences, and 3,329 deaths. These data are sufficient to provide reliable evidence to endorse or to confute the use of these drugs as adjuvant treatment for EBC. However, interpretation of such a large quantity of data is a challenging task for the average oncologist because single trials may report conflicting results.

Table 2. Sensitive Analysis for Some Relevant Groups of Studies

	DFS						OS					
	No. of Patients	Fixed Effect HR	Fixed Effect 95%CI	Random Effect HR	Random Effect 95%CI	Test of Heterogeneity P	No. of Patients	Fixed Effect HR	Fixed Effect 95%CI	Random Effect HR	Random Effect 95%CI	Test of Heterogeneity P
Combination trials*	7,540	0.84	0.76 to 0.93	0.84	0.73 to 0.96	.14	7,540	0.89	0.79 to 1.02	0.84	0.67 to 1.06	.02
Sequential trials†	15,363	0.83	0.78 to 0.88	0.83	0.78 to 0.88	.39	14,839	0.86	0.79 to 0.93	0.83	0.74 to 0.94	.09
N+ only trials‡	14,777	0.81	0.76 to 0.86	0.81	0.76 to 0.86	.47	14,777	0.83	0.76 to 0.90	0.83	0.76 to 0.90	.44

Abbreviations: DFS,disease-free survival; OS, overall survival; HR, hazard ratio; ETO; BCIRG;ECOG; CALGB; PACS; GEICAM; HeCOG; NSABP; BIG; TAXIT.
*Anglo-Celtic, BCIRG-001, ECOG E2197, ECTO, BIG2-98 (AT *v* AC).
†M.D. Anderson, CALGB B9344, PACS 01, GEICAM9906, HeCOG, NSABP B28, BIG 2-98 (A->T *v* A), NSABP B27, TAXIT 216.
‡CALGB B9344, PACS 01, BCIRG-001, GEICAM 9906,NSABP B28, BIG 2-98, TAXIT 216.

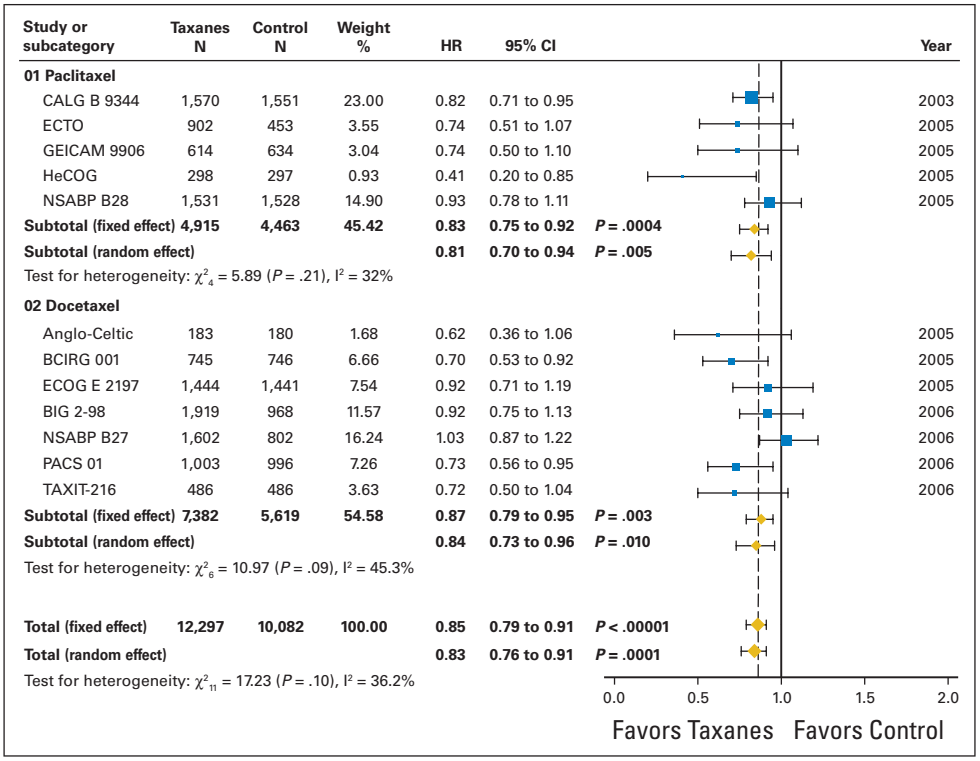

Fig 2. Meta-analysis of overall (OS). Studies are grouped based on the type of taxane used. Within each group, trials are ordered by year of reporting and by alphabetical order; squares on the hazard ratio plot are proportional to the weight of each study; weighting is based on the inverse variance method.

This is particularly true when we attempt to derive efficacy estimates in clinically relevant subgroups of patients. Indeed, this exercise can probably produce spurious results (either false-negative or false-positive) for each single trial just because of chance. In such a situation, meta-analyses may help resolve controversial issues because they give more accurate (ie, with

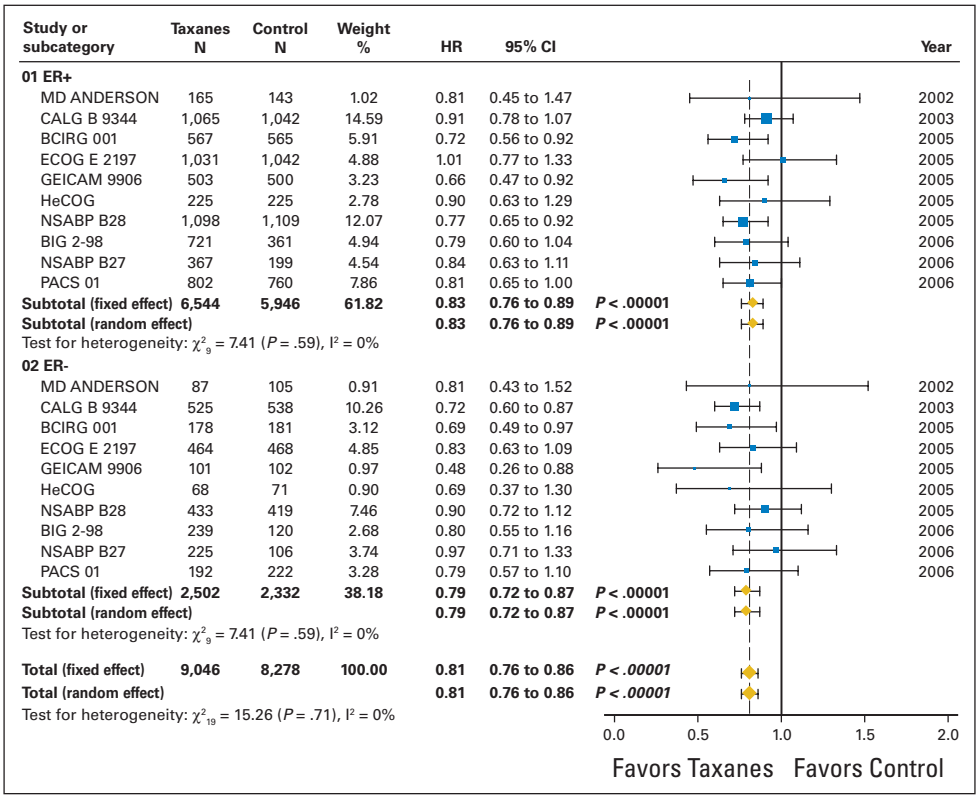

Fig 3. Meta-analysis of disease-free survival (DFS) according toestrogen receptor (ER) status. Trials are ordered by year of reporting and by alphabetical order; squares on the hazard ratio (HR) plot are proportional to the weight of each study; weighting is based on the inverse variance method.

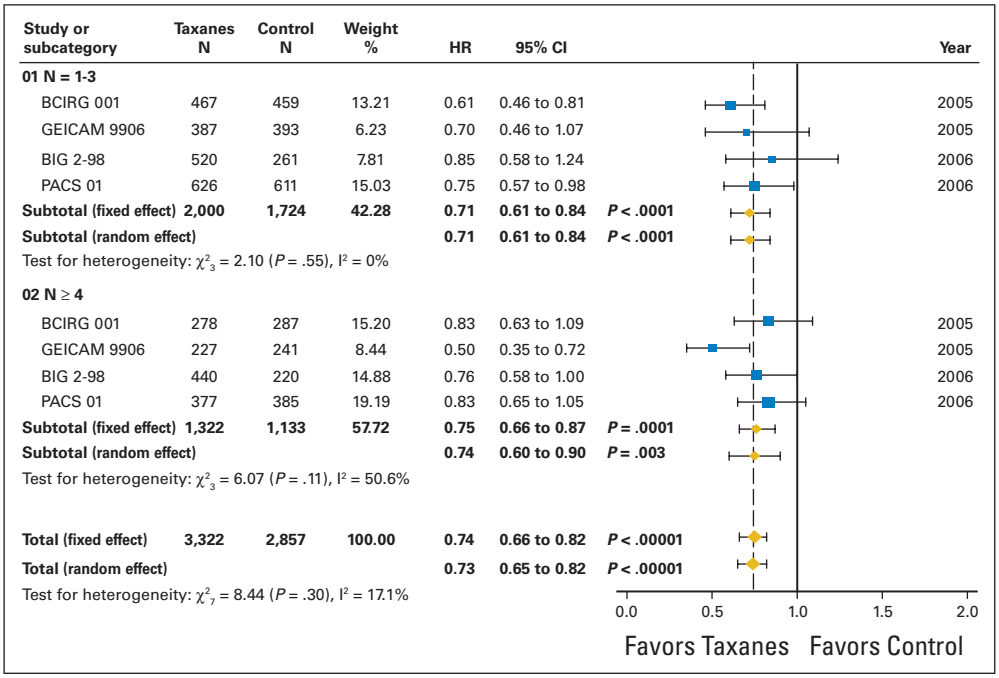

Fig 4. Meta-analysis of disease-free survival (DFS) according to nodal status. Trials are ordered by year of reporting and by alphabetical order; squares on the hazard ratio plot are proportional to the weight of each study; weighting is based on the inverse variance method.

narrower CIs) estimates of the average effect of a treatment, and also because they help identify causes of statistical heterogeneity among data (ie, single trials or specific subgroups of patients in which the observed treatment effect does not appear to be compatible with the average overall treatment effect).

In the attempt to evaluate the efficacy of the taxane-anthracycline combination in EBC cancer treatment, we extracted HRs from relevant trials and performed a meta-analysis of all available data. Specifically, we aimed to: (1) give the best estimate of the relative reduction of risk of recurrence and death; (2) give the best estimate of the magnitude of benefit in terms of absolute reduction of the risk of recurrence and death; and (3) verify whether or not such benefits remain consistent across some relevant subgroups of patients. We used data from trials published in extenso and from trials reported at

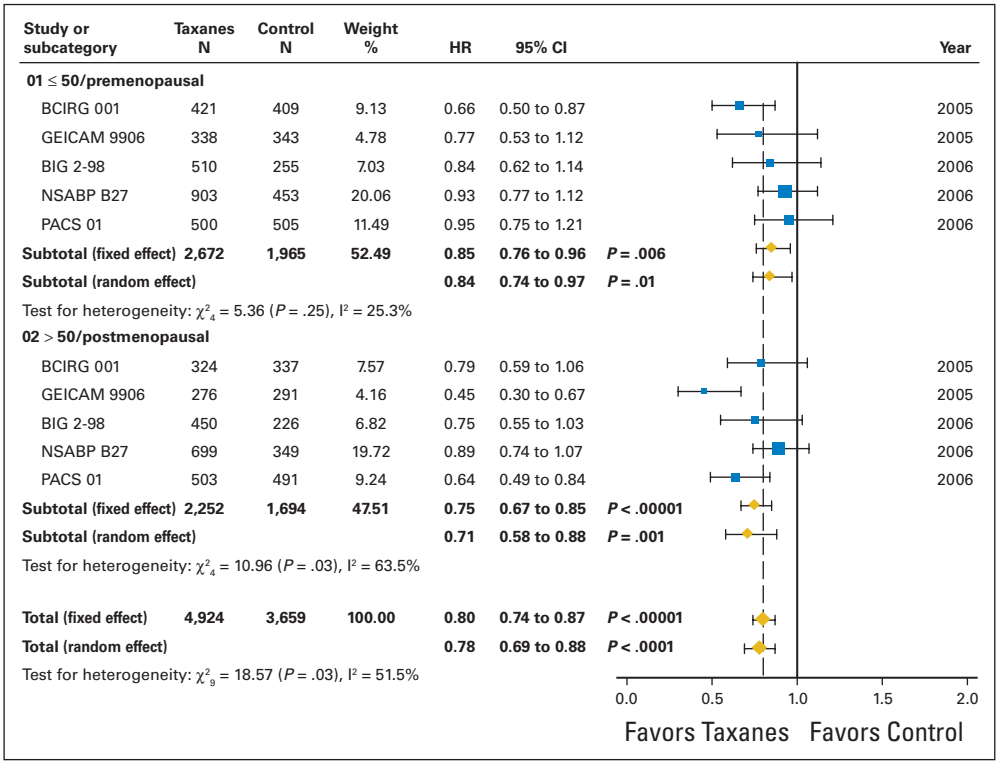

Fig 5. Meta-analysis of disease-free survival (DFS) according to age/menopausal status. Trials are ordered by year of reporting and by alphabetical order; squares on the hazard ratio (HR) plot are proportional to the weight of each study; weighting is based on the inverse variance method.

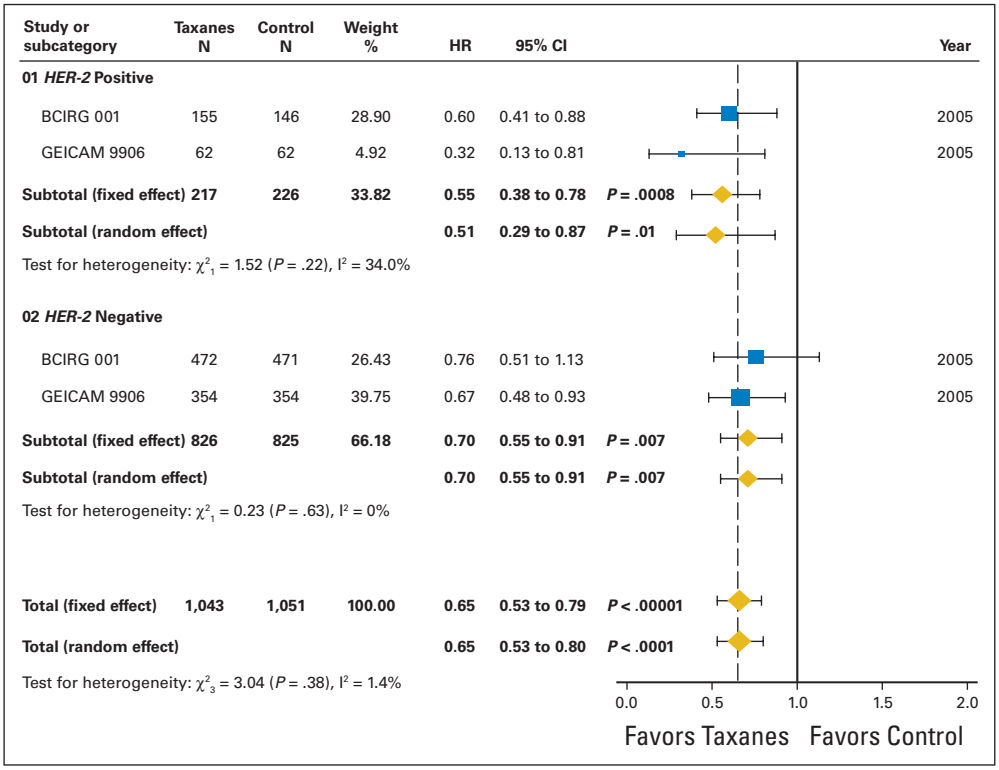

Fig 6. Meta-analysis of disease-free survival (DFS) according to HER-2 status. Trials are ordered by year of reporting and by alphabetical order; squares on the hazard ratio (HR) plot are proportional to the weight of each study; weighting is based on the inverse variance method.

meetings to minimize publication bias. The results of the Begg-Mazumdar test and of its regression equivalent indicate the absence of publication bias in our analysis.

Our meta-analysis shows that the addition of a taxane to an anthracycline-based regimen results in a statistically significant reduction of the risk of relapse (approximately 17% relative reduction) and death (approximately 15% relative reduction) for high-risk EBC patients. These benefits are also clinically relevant since they correspond to an absolute risk reduction at 5 years of approximately 5% for recurrence and 3% for death. To put these data in context, anthracyclines became the gold standard adjuvant treatment for EBC when, in the Early Breast Cancer Trialists' Collaborative Group (EBCTCG), meta-analysis showed an absolute risk reduction at 5 years of approximately 3% both for DFS and OS.[2]

A crucial issue is whether taxanes should be combined with anthracyclines or whether they should be administered after an anthracycline-based regimen. Both options have theoretical advantages and drawbacks: combination regimens require dose-reduction for both compounds, but may, in theory, exploit drug synergism. On the other hand, in sequential regimens, both compounds can be administered at optimal doses. Comparing these two approaches was not an aim of this meta-analysis, making the conclusions about this point speculative. However, due to the importance of the findings, it merits some discussion. Our sensitivity analysis shows that only sequential regimens yielded a statistically significant improvement of both DFS and OS. Conversely, with combination regimens, the trend to OS improvement was not significant, and there was statistical heterogeneity among trials. However, this result should be interpreted with caution because the test for interaction indicates that the difference between the pooled HRs of the two regimens may well be ascribed to chance. In this situation, the best HR estimate for both schedules remains that which was observed in the overall meta-analysis (Figs 1 and 2). Yet, because such results pertain to indirect comparisons, we cannot exclude that there could still be moderate but worthwhile differences in efficacy between these types of regimens, which can only be identified in a direct randomized comparison.

There is some controversy about whether taxanes produce consistent benefit across specific subgroups of patients. Single trial figures suggest that the benefits of taxanes may be lower, if not negligible, for N4+[14,17] and for ER-positive patients.[7,8,10,16,20] Other trials suggest that benefit differs between younger and older patients.[13,14,17] We were able to derive pooled estimates for these subgroups, although relevant data were available only for a subset of trials. We show that taxanes significantly reduce the risk of recurrence irrespective of ER status (ER positive *v* ER negative), nodal status (N1 to 3 *v* N4+), and age/menopausal status ($\leq$ 50 years/premenopausal *v* $>$ 50 years/postmenopausal), and that the magnitude of the relative benefit is approximately constant across such subgroups of patients. Therefore age, menopausal status, and ER status should not be used in clinical practice to identify patients who may not benefit from a taxane-based regimen.

Like all studies based on aggregated data, our meta-analysis, does not reach the level of evidence obtainable with a meta-analysis based on individual patient data (IPD) because: (1) it is impossible to determine the appropriateness of random assignment procedures; (2) trial heterogeneity can only be statistically tested, but never verified; and (3) it is not possible to do an intention-to-treat analysis because data from excluded patients cannot be retrieved. However, in our case, all authors declared their data were based on the intention-to-treat principle. In this respect, the EBCTCG is currently obtaining IPD from trials exploring the role of adjuvant taxane-based treatment. This will result in a more unbiased pooled analysis and in a finer and more

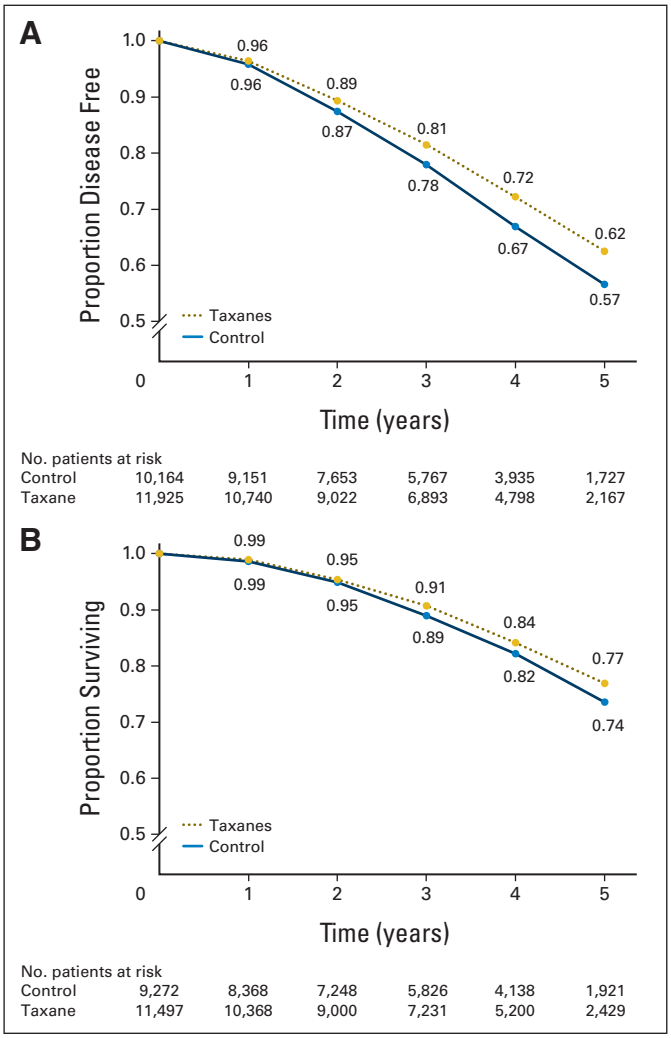

Fig 7. Pooled DFS (a) and OS (b) curves for studies included in the meta-analysis. Pts, patients.

comprehensive subgroup analysis. Nonetheless, provided a rigorous methodology is used, pooling aggregated data, as in our case, yields information that is far superior to the simple tally of positive and negative trials. As opposed to IPD meta-analysis, the simpler method we used has the advantage of speed, which is especially important when relevant clinical questions are pending. While awaiting the definitive results of the EBCTCG overview, our meta-analysis offers the most comprehensive insight into taxane-based adjuvant regimens and may help physicians and their patients worldwide to make a better informed decision regarding the most appropriate adjuvant therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

1. Goldhirsch A, Glick JH, Gelber RD, et al: Meeting highlights: International expert consensus on the primary therapy of early breast cancer 2005. Ann Oncol 16:1569-1583, 2005

2. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005

3. Chan S, Friedrichs K, Noel D, et al: Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer. J Clin Oncol 17:2341-2354, 1999

4. Paridaens R, Biganzoli L, Bruning P, et al: Paclitaxel versus doxorubicin as first-line single-agent chemotherapy for metastatic breast cancer: A European Organization for Research and Treatment of Cancer Randomized Study with cross-over. J Clin Oncol 18:724-733, 2000

5. Sledge GW, Neuberg D, Bernardo P, et al: Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: An intergroup trial (E1193). J Clin Oncol 21:588-592, 2003

6. Ghersi D, Wilcken N, Simes RJ: A systematic review of taxane-containing regimens for metastatic breast cancer. Br J Cancer 93:293-301, 2005

7. Buzdar AU, Singletary SE, Valero V, et al: Evaluation of paclitaxel in adjuvant chemotherapy for patients with operable breast cancer: Preliminary data of a prospective randomized trial. Clin Cancer Res 8:1073-1079, 2002

8. Henderson IC, Berry DA, Demetri GD, et al: Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 21:976-983, 2003

9. Bear H, Anderson S, Smith R: A randomized trial comparing preoperative (preop) doxorubicin/cyclophosphamide (AC) to preop AC followed by preop docetaxel (T) and to preop AC followed by postoperative (postop) T in patients (pts) with operable carcinoma of the breast: Results of NSABP B-27. Breast Cancer Res Treat 88:S16, 2004 (suppl 1; abstr 26)

10. Fountzilas G, Skarlos D, Dafni U, et al: Postoperative dose-dense sequential chemotherapy with epirubicin, followed by CMF with or without paclitaxel, in patients with high-risk operable breast cancer: A randomized phase III study conducted by the Hellenic Cooperative Oncology Group. Ann Oncol 16:1762-1771, 2005

11. Gianni L, Baselga J, Eiermann W: European Cooperative Trial in Operable Breast Cancer (ECTO): Improved freedom from progression (FFP) from adding paclitaxel (T) to doxorubicin (A) followed by cyclophosphamide methotrexate and fluorouracil (CMF). J Clin Oncol 23: 16s, 2005 (suppl; abstr 513)

12. Mamounas EP, Bryant J, Lembersky B, et al: Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: Results from NSABP B-28. J Clin Oncol 23:3686-3696, 2005

13. Martin M, Rodriguez-Lescure A, Ruiz A, et al: Multicenter, randomized phase III study of adjuvant chemotherapy for node positive breast cancer comparing 6 cycles of FEC90 versus 4 cycles of FEC90 followed by 8 weekly paclitaxel administrations: Interim efficacy analysis of GEICAM 9906 Trial. Breast Cancer Res Treat 94:39, 2005 (suppl; abstr 39)

14. Martin M, Pienkowski T, Mackey J, et al: Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 352:2302-2313, 2005

15. Evans TR, Yellowlees A, Foster E, et al: Phase III randomized trial of doxorubicin and docetaxel versus doxorubicin and cyclophosphamide as primary medical therapy in women with breast cancer: An anglo-celtic cooperative oncology group study. J Clin Oncol 23:2988-2995, 2005

16. Goldstein LJ, O'Neill A, Sparano J: E2197: Phase III AT (doxorubicin/docetaxel) *v* AC (doxorubicin/cyclophosphamide) in the adjuvant treatment of node-positive and high risk node-negative breast cancer. J Clin Oncol 23:16s, 2005 (suppl; abstr 512)

17. Roche H, Fumoleau P, Spielmann M, et al: Sequential adjuvant epirubicin-based and docetaxel chemotherapy for node-positive breast cancer patients: The FNCLCC PACS 01 Trial. J Clin Oncol 24:5664-5671, 2006

18. Jones SE, Savin MA, Holmes FA, et al: Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. J Clin Oncol 24:5381-5387, 2006

19. Bianco AR, De Matteis A, Manzione L, et al: Sequential Epirubicin-Docetaxel-CMF as adjuvant therapy of early breast cancer: Results of the Taxit216 multicenter phase III trial). J Clin Oncol 24:18s, 2006 (suppl; absta LBA520)

20. Crown J, Francis P, Di Leo A, et al: Docetaxel (T) given concurrently with or sequentially to anthracycline-based (A) adjuvant therapy (adjRx) for patients (pts) with node-positive (N+) breast cancer (BrCa), in comparison with non-T adjRx: First results of the BIG 2-98 Trial at 5 years median follow-up (MFU). J Clin Oncol18s, 2006 (suppl; abstr LBA519)

21. Parmar MK, Torri V, Stewart L: Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 17:2815-2834, 1998

22. Deeks JJ, Higgins JP, Altman DG: Analysing and presenting results, in Higgins JP, Green S (eds): Cochrane Handbook for Systematic Reviews of Interventions (ed 4.2.6 [updated September 2006]). Chichester, United Kingdom, John Wiley & Sons, Ltd, 2006

23. DerSimonian R, Laird N: Meta-analysis in clinical trials. Control Clin Trials 7:177-188, 1986

24. Cochran WG: The combination of estimates from different experiments. Biometrics 10:101-129, 1954

25. Higgins JP, Thompson SG: Quantifying heterogeneity in a meta-analysis. Stat Med 21:1539-1558, 2002

26. Begg CB, Mazumdar M: Operating characteristics of a rank correlation test for publication bias. Biometrics 50:1088-1101, 1994

27. Egger M, Davey SG, Schneider M, et al: Bias in meta-analysis detected by a simple, graphical test. BMJ 315:629-634, 1997

28. Deeks JJ, Altman DG, Bradburn MJ: Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis, in Egger M, Davey Smith G, Altman DG (eds): Systematic Reviews in Health Care: Meta-analysis in Context (2nd ed). London, United Kingdom, BMJ Publication Group, 2001