

Phase I clinical trial of liposomal-encapsulated doxorubicin citrate and docetaxel, associated with trastuzumab, as neo-adjuvant treatment in stages II and IIIA, HER2-overexpressing breast cancer patients. GEICAM 2003-03 study

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Background: We carried out a phase I clinical trial to establish the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) of the combination of liposome-encapsulated doxorubicin citrate (LD) and docetaxel in breast cancer patients.

Patients and methods: Patients with HER-2-overexpressing stages II and IIIA breast cancers were treated in different dose cohorts of three patients. The MTD cohort was expanded up to six patients. The patients received LD and docetaxel every 21 days, plus weekly trastuzumab, with pegfilgrastim support.

Results: A total of 20 patients were enrolled, 18 of them being assessable for toxicity and response. DLTs observed for this combination were diarrhea, fatigue, febrile neutropenia, stomatitis, myalgia, and nonneutropenic infection (pneumonia). LD 50 mg/m² and docetaxel 60 mg/m² every 21 days have been the MTD, with no episode of DLT observed. Seven patients developed left ventricular ejection fraction decline (six grade 1 and one grade 2). No interruptions of the treatment were needed as a consequence of cardiac toxicity. Pathologic complete response was achieved in eight patients (44%).

Conclusions: The MTD and recommended dose for phase II trials of LD and docetaxel are 50 and 60 mg/m², respectively. The achieved results on cardiotoxicity are promising.

Key words: Her2 positive, breast cancer, neoadjuvant chemotherapy, liposomal doxorubicine, docetaxel, trastuzumab, cardiotoxicity

introduction

Doxorubicin-based chemotherapy has been and it is yet currently the cornerstone of the breast cancer chemotherapy treatment. Cardiotoxicity is a well-known doxorubicin-associated dose-limiting toxicity (DLT) and, as a consequence of this, it is an important concern in the breast cancer chemotherapy treatment. Moreover, doxorubicin-associated cardiotoxicity may be unpredictable because it can appear at lower doses than expected in patients with a genetic predisposition as it has been demonstrated in patients carrying selected polymorphisms of the NAD(P)H oxidase and doxorubicin efflux transporters [1]. In addition, most breast cancer patients will receive radiation treatment on the chest wall which is a well-known risk factor for doxorubicin-associated cardiotoxicity, mainly if the cancer is located in the

left breast. Liposomal formulations of doxorubicin have been developed with the aim to decrease cardiotoxicity [2]. Liposome-encapsulated doxorubicin citrate (LD) (TLC D-99, Myocet™; Cephalon Inc., Frazer, PA) a nonpegylated liposome formulation of doxorubicin has been shown to offer a significant decrease in the cardiotoxicity when compared with doxorubicin while preserving doxorubicin-associated antitumor efficacy in the treatment of metastatic breast cancer patients [3–6]. Docetaxel is a member of the taxanes, a family of chemotherapy drugs which promote tubulin assembly into microtubules and inhibits their depolymerization. Docetaxel is used in both, metastatic and adjuvant setting in association with doxorubicin, improving the results of doxorubicin-based chemotherapy. Both anthracyclines and taxanes administered as single agents represent the most active groups of cytotoxic agents for the treatment of breast cancer. Up to 15%–20% of women diagnosed with early breast cancer have tumors that are HER-2

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positive which is associated with a high risk of relapse and death from metastatic disease [7–12]. Trastuzumab, a recombinant humanized mAb, directed against the extracellular domain of the HER-2, has shown to increase overall survival in HER-2-overexpressing breast cancer when associated with chemotherapy, as compared with chemotherapy alone. This improvement has been demonstrated in both settings, patients undergoing palliative chemotherapy [13–15] as well as in patients undergoing adjuvant chemotherapy [16, 17]. However, cardiotoxicity is one of the main adverse events which have been observed with trastuzumab use. Thus, the combination of trastuzumab and conventional anthracyclines has shown to carry a significant risk of cardiotoxicity [8–10, 18–22].

Neo-adjuvant chemotherapy emerged as a successful strategy to treat extensive tumors that were not candidates for surgery. This procedure was shown to be effective in prolonging overall survival [23]. As a consequence of this, neo-adjuvant treatment is being used to treat not only locally advanced breast cancer but also large, less advanced tumors. These large tumors, when treated with a neo-adjuvant approach, are optimal to evaluate efficacy of new drugs and combinations because the tumor is easily measurable and, thus, the response may be easily determined. Moreover, biologic studies can be carried out in tumor samples obtained before and after chemotherapy treatment.

We herein report the results of a phase I clinical trial attempting to establish the maximum tolerated dose (MTD) as well as the DLT of the combination of LD with docetaxel and trastuzumab, with pegfilgrastim support, as a neo-adjuvant treatment for women with HER-2-overexpressing stages II and IIIA breast cancer, paying special attention to cardiotoxicity.

patients and methods

patient population

Patients were enrolled in this trial from April 2004 to March 2006 in six centers in Spain. The inclusion criteria the patients eligible had to meet were histologically proven (breast core biopsy) stages II and IIIA breast cancer-overexpressing HER-2, confirmed by FISH carried out at a central reference laboratory; absence of systemic disease measured by bilateral mammogram, chest X-ray, abdominal computed tomography scan or ultrasound, and bone scintigraphy; a Karnofsky performance status of 80 or higher; hemoglobin ≥ 10 g/dl, absolute neutrophil count $\geq 2 \times 10^9/l$, and platelet count $\geq 100 \times 10^9/l$; adequate hepatic and renal functions; left ventricular ejection fraction (LVEF) $\geq 50\%$; no previous chemotherapy treatment (including anthracyclines or taxanes) for any previous malignancy; and no previous radiotherapy treatment for breast cancer; all patients were females at least 18 years of age considered to be fit to receive combination chemotherapy. Patients with bilateral invasive breast cancer or preexisting motor or sensorial neurotoxicity grade ≥ 2 on the basis of the National Cancer Institute—Common Toxicity Criteria version 2.0 (NCI-CTC v2.0) score system were excluded. Patients were required to provide written informed consent before inclusion in the study. The study protocol was approved by the institutional review board of each participating institution and was conducted according to the principles of the Declaration of Helsinki and the applicable guidelines for Good Clinical Practice.

drugs administration

All administered drugs were supplied by the corresponding pharmaceutical companies (LD by Cephalon, docetaxel by Sanofi-Aventis, trastuzumab by

Roche and pegfilgrastim by Amgen). Both the LD and docetaxel were administered as a 60-min i.v. infusion, escalated dose, on day 1 every 21 days. Trastuzumab was administered at fixed doses of 4 mg/kg loading dose on day 1, followed by 2 mg/kg weekly. Pegfilgrastim was administered at 6 mg, s.c., on day 2, once per cycle. It was planned to administer until a maximum of six treatment cycles, once every 21 days. The initial dosing regimen for the LD and docetaxel combination was chosen on the basis of previous studies [24, 25].

study design

The whole plan of treatment consisted of six cycles of therapy in responding patients who achieve clinical and radiological complete response, partial response or stable disease after three cycles, according to the study protocol, after which breast surgery is carried out. The starting dose of LD and docetaxel was 60 and 75 mg/m², respectively (dose level 0). Escalated dose of LD and docetaxel planning is shown in Table 1.

DLT was defined as the occurrence in the first two cycles of any of the following events: any hematological toxicity, causing >7 days delay of a subsequent cycle; febrile neutropenia; thrombopenia grades 3–4 associated with hemorrhage; and any grades 3–4 non-hematological toxicity, except nausea, vomiting and alopecia. Cohorts of three patients each were included at each dose level. If no DLT was observed in a level, an additional cohort of three patients was included in the following dose level. If any DLT was observed in one of three patients in one dose level, an additional cohort of three patients was included at the same dose level. If two or three out of three patients or two or more out of six patients at the same dose level experienced any DLT, it was considered that the MTD had been reached and the dose level right below was considered to be the MTD. Toxic effects were graded following the NCI-CTC v2.0. Although the study was not designed for such a purpose, a description of the efficacy results in terms of response rate was preplanned. Clinical and radiological responses were evaluated according to the Response Evaluation Criteria in Solid Tumors [26]. Pathological response was assessed following the Miller and Payne system, a five-point histologically grading method, which quantifies the reduction in tumor cellularity, compared with the pre-treatment tumor core biopsy [27].

results

A total of 20 patients were enrolled in this study. Two patients were removed from the study. The first patient, at –2 level (LD 50/Doc 70), experienced a chronic hepatitis C reactivation after cycle 1 and she was withdrawn from the study. The second one was enrolled in the study but she never received the treatment due to internal problems with the Hospital Pharmacy Department. A total of 18 patients were assessable for toxicity as well as for efficacy. Demographic patient characteristics are listed in Table 2.

toxicity

As unexpectedly DLTs appeared in two of three patients in the starting dose level (LD 60/Doc 75, dose level 0) (grade 3

Table 1. Dose-escalation process planned according to protocol

[LD (mg/m ²)/docetaxel (mg/m ²)]
60/75
70/70
70/75
75/75

LD, liposome-encapsulated doxorubicin citrate.

Table 2. Baseline patients' characteristics

Characteristics	n = 19
Age in years, median (range)	45.3 (31–63)
Menopausal state (n, %)	
Premenopausal	12 (63.2%)
Postmenopausal	7 (36.8%)
Performance status (Karnofsky)	
100	17 (89.5%)
90	2 (10.5%)
LVEF %, median (range)	61 (51–74)
Tumor stage (n, %)	
IIa	6 (31.6%)
IIb	7 (36.8%)
IIIa	6 (31.6%)
Tumor histology (n, %)	
Ductal	17 (89.4%)
Lobular	1 (5.3%)
Carcinoma NOS	1 (5.3%)
Histology grade (n, %)	
Unknown	1 (5.3%)
Grade 1	2 (10.5%)
Grade 2	9 (47.4%)
Grade 3	7 (36.8%)
Hormone (estrogen and progesterone) receptors (n, %)	
Negative (ER– and PR–)	7 (36.8%)
Positive [(ER+ and PR+) or (ER– and PR+) or (ER+ and PR–)]	12 (63.2%)

LVEF, left ventricular ejection fraction; NOS, Not otherwise specified; ER, estrogen receptor; PR, progesterone receptor.

diarrhea in one patient and a combination of grade 3 diarrhea and grade 3 stomatitis in another patient), a deescalated dose drug administration was carried out instead of the escalated one that had been initially planned. Deescalated dose of LD and docetaxel, actually carried out, is shown in Table 3.

Diarrhea and febrile neutropenia were the most commonly observed toxic effects at the first dose levels and were the main DLT. All the DLTs are listed in Table 4. At the MTD, there were no episodes of DLT. A total of three patients [two in level 0 and one in –1 level (LD 55/Doc 75)] experienced non-hematological toxicity that resulted in dose reduction during cycle 1. A total of three patients [one in –2 level and two in –3 level (LD 50/Doc 65)] experienced non-hematological toxicity that resulted in dose reduction during cycle 2. Four patients had treatment delays. The first patient due to grade 2 liver function test abnormalities, the second patient due to grade 2 rash, the third patient due to infection without neutropenia associated with grade 2 rash, and the fourth patient due to arthralgias associated with epigastric pain. One additional patient missed days 8 and 15 trastuzumab treatment secondary to grade 2 diarrhea, fatigue, myalgia, urinary infection, abdominal pain, and cephalalgia.

One patient developed grade 3 infection without neutropenia (an episode of pneumonia due to *Klebsiella pneumoniae*). Grade 3–4 toxic effects of all the cycles of treatment are listed in Table 5. Overall, six patients did not complete treatment secondary to toxicity.

Table 3. Dose-deescalation process conducted in practice

[LD (mg/m ²)/docetaxel (mg/m ²)]
60/75 (level 0)
55/75 (level –1)
50/70 (level –2)
50/65 (level –3)
50/60 (level –4)

LD, liposome-encapsulated doxorubicin citrate.

Six patients developed grade 1 LVEF decline [two in –2 level, one in –3 level, and three in –4 level (LD 50/Doc 60)] and one patient (–1 level) developed a grade 2 LVEF decline.

Two consecutive cohorts of three patients were included in a dose level of LD 50 mg/m² and docetaxel 60 mg/m² and with no episode of DLT. Thus, LD (50 mg/m²) and docetaxel (60 mg/m²) in combination with trastuzumab with pegfilgrastim support was the recommended dose for phase II trials.

efficacy

A total of 18 patients were assessable for response. Evaluation of response has been carried out after three and six cycles of treatment. All patients underwent surgery after neo-adjuvant treatment. A clinical response has been obtained in 14 patients (78%), eight of which (44%) with a clinical complete response. A radiological response has been obtained in 13 patients (72%) including eight radiological complete responses (44%). In 14 patients (78%), conservative surgery was carried out and four patients (22%) underwent a mastectomy. Margins of resection were negative in all cases and seven patients had positive lymph nodes, including one patient with five affected nodes. Pathological response was evaluated according to the Miller and Payne system. A complete disappearance of breast cancer cellular elements in the resected specimen was observed in eight patients (44%). Pathological complete response (pCR) in both breast and axillary lymph nodes was achieved in six patients (33%).

discussion

This phase I trial is the first study reported of a regimen combining LD, docetaxel, and trastuzumab in breast cancer patients. This regimen has been administered within a dose-intense regimen which needed the prophylactic use of pegfilgrastim because a rate of febrile neutropenia >20% was expected.

In our study, the starting dose level (LD 60 mg/m² and docetaxel 75 mg/m², dose level 0) has proved to be too toxic, resulting in two of three patients experiencing a DLT (grade 3 diarrhea in one patient and a combination of grade 3 diarrhea and grade 3 stomatitis in another patient). Thus, deescalation of the dose in the subsequent cohorts had to be carried out instead of the planned classical phase I escalation method. An unexpected high rate of grade 3 diarrhea has been observed (another patient in the –3 level has experienced it). Diarrhea is not dose limiting for either LD or docetaxel when used as

Table 4. Dose-limiting toxic effects

Dose level	No. of patients (cycles)	DLTs (cycle 1)	DLTs (cycle 2)
Level 0 (LD 60/Doc75)	3 (15)	One diarrhea and stomatitis (grade 3); one diarrhea (grade 3)	0
Level -1 (LD 55/Doc 75)	3 (13)	One febrile neutropenia (grade 3); one fatigue and myalgias (grade 3)	
Level -2 (LD 50/Doc 70)	4 ^a (15)	Two febrile neutropenia (grade 3)	One fatigue (grade 4)
Level -3 (LD 50/Doc 65)	3 (14)	0	One infection without neutropenia (grade 3) [pneumonia]; one diarrhea (grade 3)
Level -4 (LD 50/Doc 60)	6 (36)	0	0

^aOne patient was removed from the study after cycle 1, due to a chronic C hepatitis reactivation. DLTs, dose-limiting toxicities; LD, liposomal-encapsulated doxorubicin citrate; Doc, docetaxel.

Table 5. Grade 3/4 toxic effects

Dose level 0 (60/75)	<i>n</i> = 3	Dose level -1 (55/75)	<i>n</i> = 3
Hot flashes G3	1	Leukopenia G3	1
Diarrhea G3	2	Leukopenia G4	1
Nauseas G3	1	Neutropenia G4	1
Mucositis G3	1	Thrombocytopenia G3	1
Liver function test G3	1	Febrile neutropenia G3	1
Infection without neutropenia G3	1	Diarrhea G3	1
		Fatigue G3	1
		Myalgias G3	1
		Abdominal pain G3	1
Dose level -2 (50/70)	<i>n</i> = 4	Dose level -3 (50/65)	<i>n</i> = 3
Leukopenia G3	3	Leukopenia G3	1
Neutropenia G3	1	Neutropenia G3	1
Febrile neutropenia G3	2	Lymphopenia G3	1
Fatigue G3	1	Constipation G3	1
Fatigue G4	1	Diarrhea G3	1
		Infection without neutropenia G3	1
Dose level -4 (50/60)	<i>n</i> = 6		
Neutropenia G4	1		
Neutropenia G3	1		

G3, grade 3; G4, grade 4.

monotherapy. A possible explanation for this observation may be the combination with trastuzumab. Delaying cycles as a consequence of prolonged afebrile neutropenia has not been necessary. However, febrile neutropenia has been observed as DLT in three patients despite prophylactic use of pegfilgrastim, one in the -1 level (LD 55/Doc 75) and two in the -2 level (LD 50/Doc 70).

The results on cardiotoxicity should be highlighted. Only one patient experienced grade 2 decline of the LVEF (at -1 level). At this level, LD has been administered at 55 mg/m², 5 mg/m² higher than the dose at MTD. In addition, six more patients experienced grade 1 LVEF decline. All the patients experiencing these cardiac events remained asymptomatic and thus no cases of clinical congestive heart failure have been observed. In addition, no cases of early drop out from the study because of cardiotoxicity have been observed, either. These results are important for a combination of an anthracycline with trastuzumab, a well-known cardiotoxic combination. In

addition, many, if not all, of these patients will continue to receive additional trastuzumab treatment. However, due to the small sample size of our study, we consider that cardiotoxicity results should be confirmed in future trials, such as a phase II trial we are now conducting.

Changing conventional doxorubicin for another less cardiotoxic anthracycline in an anthracycline-taxane-trastuzumab regimen in the neo-adjuvant treatment of breast cancer is a strategy which has previously been reported with mixed results. In a phase II trial, epirubicin was used associated with docetaxel and trastuzumab, showing an exceeding rate of cardiotoxicity for this combination which led to stop the enrollment of patients in the trial [28]. Trastuzumab, paclitaxel, and epirubicin in combination have recently been evaluated in the neo-adjuvant setting [29]. Despite the small sample size of this trial, its results indicated that the addition of trastuzumab to chemotherapy significantly increased pCR rates without incurring the risk of clinical congestive heart failure.

The first data on the association of LD and trastuzumab were presented at the ASCO meeting in 2002. In this phase I–II study, patients with metastatic breast cancer which had previously been treated with anthracyclines received treatment with LD and trastuzumab. Along with a global response rate of 58%, the key finding of the trial was the excellent cardiac tolerability: only two patients experienced a reversible decline in the LVEF [30]. The results of a recent phase II study which investigated the combination of LD with weekly trastuzumab and paclitaxel in patients with HER2-positive locally advanced or metastatic breast cancer reported high response rates in both groups of patients (100% and 96% respectively), with acceptable toxicity and no association with symptomatic cardiac heart failure [31].

Results on efficacy should be considered only as exploratory. Breast cancer patients who achieve a pCR after neo-adjuvant chemotherapy have an increased overall survival as compared with the patients who did not [32]. We consider that the rate of pCR in our study, with ~33% of patients having a pCR in both breast and axillary lymph nodes, is promising and warrants further investigation.

In conclusion, we have demonstrated that the MTD and recommended doses for phase II trials of LD and docetaxel in combination when administered with prophylactic pegfilgrastim and standard dose trastuzumab are LD 50 mg/m² and docetaxel 60 mg/m² every 21 days. DLTs for this combination are diarrhea, fatigue, febrile neutropenia, stomatitis, myalgia, and nonneutropenic infection. No DLTs have been observed at the MTD. Cardiotoxicity and efficacy results, although nonconclusive, have been promising. A phase II trial looking for the efficacy of this regimen in stage II and IIIA breast cancer patients is ongoing. The enrollment period of this trial has ended after 59 patients have been included and treated at the recommended dose for phase II trials (LD 50/Doc 60). We are now ending the statistical analyses of this trial.

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references

1. Wojnowski L, Kulle B, Schirmer M et al. NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. *Circulation* 2005; 112: 3754–3762.
2. Rahman AM, Yusuf SW, Ewer MS. Anthracycline-induced cardiotoxicity and the cardiac-sparing effect of liposomal formulation. *Int J Nanomed* 2007; 2: 567–583.
3. Janknecht R. Liposomal formulations of cytotoxic drugs. *Support Care Cancer* 1996; 4: 298–304.
4. Harris L, Batist G, Belt R et al. Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer* 2002; 94: 25–36.
5. Batist G, Ramakrishnan G, Rao CS et al. Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. *J Clin Oncol* 2001; 19: 1444–1454.
6. Batist G, Harris L, Azamia N et al. Improved anti-tumor response rate with decreased cardiotoxicity of non-pegylated liposomal doxorubicin compared with conventional doxorubicin in first-line treatment of metastatic breast cancer in patients who had received prior adjuvant doxorubicin: results of a retrospective analysis. *Anticancer Drugs* 2006; 17: 587–595.
7. Slamon D, Eiermann W, Robert N et al. Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab with docetaxel, carboplatin and trastuzumab in HER2 positive early breast cancer patients: BCIRG 006 study. *Breast Cancer Res Treat* 2005; 94: S5.
8. Tan-Chiu E, Yothers G, Romond E et al. Assessment of cardiac dysfunction in a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel, with or without trastuzumab as adjuvant therapy in node-positive, human epidermal growth factor receptor 2-overexpressing breast cancer: NSABP B-31. *J Clin Oncol* 2005; 23: 7811–7819.
9. Pérez EA, Suman VJ, Davidson NE et al. Interim cardiac safety analysis of NCCTG N9831 intergroup adjuvant trastuzumab trial. *J Clin Oncol (Meeting Abstracts)* 2005; 23: 17s.
10. Romond EH, Perez EA, Bryant J et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005; 353: 1673–1684.
11. Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005; 353: 1659–1672.
12. Smith I, Procter M, Gelber RD et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomized controlled trial. *Lancet* 2007; 369: 29–36.
13. Vogel CL, Cobleigh MA, Tripathy D et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002; 20: 719–726.
14. Slamon DJ, Leyland-Jones B, Shak S et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344: 783–792.
15. Marty M, Cognetti F, Maraninchi D et al. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 2005; 23: 4265–4274.
16. Romond EH, Perez EA, Bryant J et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005; 353: 1673–1684.
17. Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005; 353: 1659–1672.
18. Pegram M, Hsu S, Lewis G et al. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. *Oncogene* 1999; 18: 2241–2251.
19. Baselga J, Norton L, Albanell J et al. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res* 1998; 58: 2825–2831.
20. Pietras RJ, Pegram MD, Finn RS et al. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. *Oncogene* 1998; 17: 2235–2249.
21. Baselga J, Seidman AD, Rosen PP et al. HER2 overexpression and paclitaxel sensitivity in breast cancer: therapeutic implications. *Oncology (Williston Park)* 1997; 11 (Suppl): 43–48.
22. Perez EA, Suman VJ, Davidson NE et al. Cardiac safety analysis of doxorubicin and cyclophosphamide followed by paclitaxel with or without trastuzumab in the North Central Cancer Treatment Group N9831 Adjuvant Breast Cancer Trial. *J Clin Oncol* 2008; 10: 1231–1238.
23. De Lena M, Varini M, Zucali R et al. Multimodal treatment for locally advanced breast cancer. Result of chemotherapy-radiotherapy versus chemotherapy-surgery. *Cancer Clin Trials* 1981; 4: 229–236.

24. Possinger K, Krocker J, Fritz J et al. Primary chemotherapy for locally advanced breast cancer (LABC) with gemcitabine (G) as prolonged infusion, liposomal doxorubicin (M) and docetaxel (T): results of a phase I trial. *Proceedings ASCO* 2002 A-1971.
25. Marty M, Laurence V, Cottu P et al. A phase I dose escalation study of non-pegylated liposomal doxorubicin (M) and docetaxel (D) in patients with previously untreated advanced breast cancer. *Ann Oncol* 2002; 13 (Suppl 5): 55.
26. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; 92: 205–216.
27. Ogston KN, Miller ID, Payne S et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast* 2003; 12: 320–327.
28. Venturini M, Bighin C, Monfardini S et al. Multicenter phase II study of trastuzumab in combination with epirubicin and docetaxel as first-line treatment for HER2-overexpressing metastatic breast cancer. *Breast Cancer Res Treat* 2006; 95: 45–53.
29. Buzdar AU, Valero V, Ibrahim NK et al. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. *Clin Cancer Res* 2007; 13: 228–233.
30. Theodoulou M, Campos SM, Batist G et al. TLC D99 (D, Myocet) and Herceptin (H) is safe in advanced cancer (ABC): final cardiac safety and efficacy analysis. *Proceedings Am Soc Clin Oncol* 2002; 21: 55a.
31. Cortés J, Climent M, Lluch A et al. Updated results of a phase II study (M77035) of Myocet combined with weekly Herceptin and paclitaxel in patients with HER2-positive locally advanced or metastatic breast cancer (LABC/MBC). *Breast Cancer Res Treat* 2004; 88 (Suppl 1): S125 (Abstr 3041).
32. Rastogi P, Anderson SJ, Bear HD et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. *J Clin Oncol* 2008; 26: 778–785.