original article

Annals of Oncology 22: 74–79, 2011
doi:10.1093/annonc/mdq317
Published online 5 July 2010

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

A. Antón[1]*, A. Ruiz[2], A. Plazaola[3], L. Calvo[4], M. A. Seguí[5], A. Santaballa[6], M. Muñoz[7], P. Sánchez[8], A. Miguel[9], E. Carrasco[10], J. Lao[1], J. Camps[2], J. Alfaro[3], S. Antolín[4] & M. C. Cámara[10]

[1]*Oncology Department, Hospital Universitario Miguel Servet, Zaragoza;* [2]*Oncology Department, Instituto Valenciano de Oncología, Valencia;* [3]*Oncology Department, Onkologikoa, San Sebastián;* [4]*Oncology Department, Complejo Hospitalario Universitario de A Coruña, A Coruña;* [5]*Oncology Department, Consorci Sanitari Parc Taulí, Sabadell;* [6]*Oncology Department, Hospital Universitario La Fe, Valencia;* [7]*Oncology Department, Hospital Clínic i Provincial, Barcelona;* [8]*Oncology Department, Complejo Hospitalario de Jaén, Jaén;* [9]*Oncology Department, Althaia-Xarxa Assistencial de Manresa, Manresa;* [10]*Staff members of GEICAM, Madrid, Spain*

Received 31 March 2010; accepted 21 April 2010

Background: We previously reported a phase I trial of liposome-encapsulated doxorubicin citrate (LD), docetaxel and trastuzumab as neoadjuvant in stages II and IIIA human epidermal growth factor receptor 2-overexpressing breast cancer patients. This study evaluates the efficacy of this regimen in a phase II trial.

Patients and methods: Patients were treated with LD 50 mg/m^2 and docetaxel 60 mg/m^2 every 21days associated with standard trastuzumab dose and pegfilgrastim support.

Results: Fifty-nine patients were enrolled; median age: 48 years (range 24–71 years); premenopausal patients: 36 (61%); 19 patients (32%) presented stage IIIA disease and 40 patients (67%) stage II; histological grades 2–3 tumors: 50 patients (84%) and estrogen receptor–progesterone receptor negative: 28 patients (47%). In all, 27% achieved a pathological complete response in breast and axilla (grade 5—Miller and Payne classification); 15% of patients achieved grade 4. Clinical and radiological response rates were 86% and 81%, respectively. Forty-two patients (71%) underwent breast-conserving surgery. The main grades 3–4 toxic effects were non-febrile neutropenia (29%) and fatigue (8%). Grade 2 left ventricular ejection fraction decline was observed in nine patients. No congestive heart failure was observed.

Conclusions: LD plus docetaxel combination associated with trastuzumab as neoadjuvant is active in breast cancer and entails a favorable cardiotoxicity profile. This regimen is a new treatment option in these patients.

Key words: cardiotoxicity, docetaxel, HER2-overexpressing breast cancer, liposomal doxorubicin, trastuzumab

introduction

Neoadjuvant chemotherapy (NAC) has been the preferred treatment of unresectable stage III (also known as locally advanced) breast cancer patients. A multimodality approach, including NAC, surgery and radiation, with or without additional hormonal treatment, is necessary to treat these patients [1]. This strategy has shown to be effective, improving overall survival in this setting.

NAC has shown high clinical and pathologically documented response (pathological response) rates and, in consequence, its use is increasing in the treatment of large or borderline resectable tumors. Biological advantages for a preoperative strategy, even in smaller tumors, have been shown in preclinical models [2]. Both *in vivo* chemosensitivity testing and downstaging of primary tumor [thus allowing for an increased rate of breast-conserving surgery (BCS)] are the main advantages of using NAC in these patients. More importantly, it can be stated that pathological complete response (pCR) is a proper surrogate end point for disease-free survival and overall survival [3, 4]. Thus, studying new regimens with the aim of increasing pCR rates is becoming the main objective of

*Correspondence to: Dr A. Antón, Oncology Department, Hospital Universitario Miguel Servet, Paseo Isabel La Católica 1, 50009 Zaragoza, Spain. Tel: +34-976765560; Fax: +34-976359268; E-mail: aantont@gmail.com

the NAC strategy in operable tumors, in order to turn into longer survival.

Up to 20% of breast tumors are human epidermal growth receptor 2 (HER2) positive. Trastuzumab, a recombinant humanized monoclonal antibody, directed against the extracellular domain of the HER2 receptor, increases overall survival in HER2-overexpressing breast cancer in both advanced and early breast cancer patients. However, trastuzumab is cardiotoxic and increases the anthracycline-associated cardiotoxicity, when used in combination regimens [5].

Liposomal doxorubicin formulations have been developed in an attempt to improve its cardiotoxicity profile [6]. Liposome-encapsulated doxorubicin citrate (LD) (TLC D-99, Myocet®; Cephalon Inc., Frazer, PA), a non-pegylated liposome formulation, is less cardiotoxic than conventional doxorubicin, while preserving its efficacy when used in metastatic breast cancer patients [7–9].

We reported previously the results of a phase I clinical trial of an LD, docetaxel and trastuzumab combination regimen used as an NAC in stages II and IIIA HER2-overexpressing breast cancer patients [10]. The recommended doses for phase II trials with this combination, when administered with prophylactic pegfilgrastim support and trastuzumab standard dose, were LD 50 mg/m^2 and docetaxel 60 mg/m^2 every 21 days. Cardiotoxicity results were promising; only 1 of 19 patients experienced grade 2 left ventricular ejection fraction (LVEF) decline and no clinical congestive heart failure (CHF) was observed. Exploratory efficacy results were also interesting with 33% pCR rate, in both tumor and axilla.

The aforementioned phase I clinical trial laid the foundation of the phase II clinical trial we are reporting now.

patients and methods

patient population

Females with histologically proven (breast core biopsy) stages II and IIIA breast cancer and HER2 overexpression, confirmed by FISH carried out in a central laboratory, were included in this study. Patients were required to be >18 years; have a Karnofsky performance status (PS) ≥80; hemoglobin ≥10 g/dl, absolute neutrophil count ≥2 × 10^9/l and platelet count ≥100 × 10^9/l; adequate hepatic and renal functions; LVEF ≥50% and no previous chemotherapy or radiotherapy for breast cancer. Patients with either bilateral invasive breast cancer or preexisting 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. The study 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 guidelines for Good Clinical Practice. All patients provided written informed consent before entering the study.

drugs administration

Both LD (50 mg/m^2) and docetaxel (60 mg/m^2) were administered as a 60-min i.v. infusion on day 1 every 21 days. Trastuzumab, 4 mg/kg, was administered on day 1 (loading dose), followed by 2 mg/kg weekly. Pegfilgrastim was administered s.c. at a dose of 6 mg on day 2. These drugs were administered for six cycles unless disease progression or unacceptable toxicity occurs. Thereafter, patients without disease progression were scheduled to undergo breast surgery (mastectomy or conservative treatment) plus lymphadenectomy.

The main planned dose modifications were as follows: a reduction of 10 mg/m^2 in LD and docetaxel doses in case of hematological toxicity (platelets < 10 × 10^9/l lasting ≥3 days, absolute neutrophil count < 0.5 × 10^9/l lasting ≥7 days or febrile neutropenia) or grade ≥3 diarrhea, stomatitis and liver toxicity. Patient discontinuation was planned in the case of grade ≥2 cardiac toxicity.

study design

The primary end point of this study was to determine the pCR rate achieved in these patients. Secondary objectives were safety, with a special focus on cardiotoxicity, breast-conserving treatment rate, the determination of clinical and radiological response rates and axillary disease after surgery.

patient assessments

Before study entry, all patients had a breast and axillary disease assessment by physical examination (PE) and diagnostic breast imaging (mammogram or magnetic resonance imaging), Karfnosky PS evaluation, core biopsy, complete blood cell count, serum chemistry and LVEF measurement (by echocardiography or multiple-gated acquisition scan).

Pathologic response was assessed at surgery following the Miller and Payne classification (Figure 1), a five-point histological grading method, which quantifies the reduction in tumor cellularity, as compared with the pre-treatment tumor core biopsy [11].

Clinical response was evaluated after cycles 3 and 6, by PE and breast imaging, according to RECIST.

Toxic effects were graded following the NCI–CTC v2.0 score system, and the worst toxicity per cycle was recorded. Complete blood cell count was carried out weekly and serum chemistry every 21 days. LVEF was evaluated every two cycles. Relative dose intensity was defined as the percentage of the planned dose intensity (mg/kg/week for trastuzumab; mg/m^2/week for both LD and docetaxel), which the patients actually received.

statistical considerations

Simon's method was used to calculate the sample size with null hypothesis (H0) of pCR rate equal to 11% against the alternative hypothesis (H1) that pCR rate is not equal to 25%. Fifty-three assessable patients were required to be recruited in this study; considering a 10% dropout, 59 patients were needed.

The analyses have been carried out with the Statistical Package for Social Sciences (SPSS) 11.0 software. All hypotheses have been tested at an alpha level of 0.05.

results

patient characteristics

A total of 53 patients were enrolled in this trial in 12 centers in Spain. We report here the efficacy and safety results of these 53 patients, together with 6 additional patients from the phase I, treated with the final recommended phase II doses.

All 59 patients were assessable for safety and efficacy (in an intention-to-treat analysis). Median age was 48 years (range 24–71 years), 36 patients (61%) were premenopausal, 19 patients (32%) presented with stage IIIA disease and 40 patients (67%) with stage II, median tumor size was 3.4 cm (range 1–12 cm), 50 patients (84%) had histological grades 2–3 tumors and 28 patients (47%) were estrogen receptor–progesterone receptor negative. Baseline demographic and disease characteristics are listed in Table 1.

Four patients discontinued the trial due to adverse events: grade 3 fatigue plus febrile neutropenia on cycle 2, grade 2 allergy to LD on cycle 5, decrease in LVEF <50% on cycle 4 and grade 4 pulmonary thromboembolism on cycle 4.

PRIMARY SITE RESPONSE	
Grade 1	Some alteration to individual malignant cells but no reduction in overall numbers as compared with the pretreatment core biopsy
Grade 2	A minor loss of invasive tumor cells but overall cellularity still high
Grade 3	A moderate reduction in tumor cells up to an estimated 90% loss
Grade 4	A marked disappearance of invasive tumor cells such that only small clusters of widely dispersed cells could be detected
Grade 5	No invasive tumor, ie, only in-situ disease or tumor stroma remained
AXILLARY LN RESPONSE	
N-A	True axillary N negative
N-B	Axillary N positive and no therapeutic effects
N-C	Axillary N positive but evidence of partial pathologic response
N-D	Initially axillary N positive but converted to N negative after primary systemic therapy

Abbreviation: N, node

Figure 1. Miller and Payne classification system.

Table 1. Patient baseline demographic and disease characteristics

Median age (range), years	48 (24–71)
Menopausal status, *n* (%)	
Premenopausal	36 (61)
Postmenopausal	23 (39)
Karnofsky performance status, *n* (%)	
80	1 (2)
90	10 (17)
100	48 (81)
Stage, *n* (%)	
IIA	15 (25)
IIB	25 (42)
IIIA	19 (32)
Hormone receptor status[a], *n* (%)	
Negative	28 (47)
Positive	31 (52)
Histological type, *n* (%)	
Ductal	42 (71)
Lobular	16 (27)
Carcinoma NOS	1 (2)
Histological grade, *n* (%)	
1	1 (2)
2	25 (42)
3	25 (42)
Unknown	8 (13)
Median radiological size (range), cm	3.4 (1–12)
Median baseline LVEF (range), %	64 (51–79)

[a]Hormone receptor status is a combined evaluation of estrogen and progesterone; only considered negative if both are negative.
NOS, not otherwise specified; LVEF, left ventricular ejection fraction.

efficacy

Seventeen patients [29%, 95% confidence interval (CI) 17.2–40.4] achieved a pCR in the breast (grade 5 according to Miller and Payne classification); all of them, but one (27%, 95% CI 15.8–38.4), were also free of invasive tumor in the axilla (Table 2). Major pathological response (Miller and Payne grades 4 + 5) without nodal involvement was seen in 25 patients (42%, 95% CI 29.4–54.6).

The clinical response rates, evaluated by PE and breast imaging, were 86% and 81%, respectively (Table 3); no patients progressed during treatment.

All patients underwent surgery; breast conservation was possible in 42 patients. Axillary lymphadenectomy was carried out in all but one patient, in which sentinel node procedure was done resulting in a tumor-free axilla. The median number of nodes retrieved at surgery was 16 (1–29).

toxicity

The most frequent grades 3–4 toxicity was neutropenia, which was observed in 17 patients (29%); 3 of them suffered febrile neutropenia. Grade 3 non-hematologic toxicity was infrequent with fatigue in five patients, nausea in three, diarrhea in three and stomatitis in one. Table 4 summarizes grades 3–4 adverse events.

Symptomatic congestive cardiac failure (NCI–CTC v2.0 grades 3–4 LVEF decline) was not observed. Median LVEF was 64% (range 51%–79%) at baseline and 61% (range 46%–86%) at the end of treatment. Five patients had grade 2 LVEF decline during treatment, only one of them discontinued due to this reason (LVEF = 46% after cycle 4). In the post-treatment final evaluation, four patients had an LVEF decline >20%, three of them with a final LVEF ≥50% and one with 49%. Table 5 summarizes LVEF.

dose administration

Docetaxel dose was reduced in two patients because of febrile neutropenia and grade 3 diarrhea. LD dose was only reduced in one patient due to febrile neutropenia. Eight patients suffered dose delays due to several minor non-hematologic toxic effects.

Median relative dose intensities were 98.88%, 99.14% and 99.36% for LD, docetaxel and trastuzumab, respectively.

Table 2. Pathological response (Miller and Payne classification)

Axilla	Tumor				
	G1	G2	G3	G4	G5
N-A	1	6	6	3	6
N-B	0	3	0	1	1
N-C	1	4	2	5	0
N-D	1	1	2	6	10
Total	3	14	10	15	17

G, grade; N, node.

Table 3. Clinical and radiological response

	After three cycles		After six cycles	
	Clinical, *n* (%)	Radiological, *n* (%)	Clinical, *n* (%)	Radiological, *n* (%)
CR	15 (25)	6 (10)	31 (52)	20 (34)
PR	31 (52)	32 (54)	20 (34)	28 (47)
ORR	46 (78)	38 (64)	51 (86)	48 (81)
SD	8 (14)	12 (20)	2 (3)	6 (10)
ND	4 (7)	8 (13)	3 (5)	2 (3)
NA	1 (2)[a]	1 (2)[a]	3 (5)[a]	3 (5)[a]

[a]Three patients did not complete treatment due to adverse events (one of them before cycle 3).
CR, complete response; PR, partial response; ORR, overall response rate; SD, stable disease; ND, not done; NA, not applicable.

Table 4. Grades 3–4 NCI–CTC v2.0 adverse events ($N = 59$)

Adverse event	*n* (%)
Hematologic	
Anemia	0 (0)
Neutropenia	17 (29)
Thrombocytopenia	3 (5)
Febrile neutropenia	3 (5)
Non-hematologic	
Allergy/hypersensitivity reaction	1 (2)
Alopecia[a]	40 (68)
Constipation	1 (2)
Diarrhea	3 (5)
Dyspnea	1 (2)
Fatigue	5 (8)
Hypertension	1 (2)
Nausea + vomiting	4 (7)
Stomatitis	1 (2)
Syncope	1 (2)
Thrombosis/embolism	1 (2)

[a]Grade 2.
NCI–CTC v2.0, National Cancer Institute—Common Toxicity Criteria version 2.0.

postoperative systemic treatment

All patients continued treatment with trastuzumab after surgery. Hormonal therapy was administered in 30 patients (50.8%).

Table 5. Left ventricular ejection fraction ($N = 59$)

Maximum NCI–CTC v2.0 grade	During treatment ($n = 58$)	End of treatment ($n = 54$)
Grade 1 (asymptomatic decline of resting ejection fraction of ≥10% but <20% of baseline value; shortening fraction ≥24% but <30%)	18 (31%)[a]	14 (26%)
Grade 2 (asymptomatic but resting ejection fraction below LLN for laboratory or decline of resting ejection fraction ≥20% of baseline value; <24% shortening fraction)	5 (9%)[b]	4 (7%)
Grade 3 (CHF responsive to treatment)	0 (0.0%)	0 (0.0%)
Grade 4 (severe or refractory CHF or requiring intubation)	0 (0.0%)	0 (0.0%)

[a]Six of them were also grade 1 and two became grade 2 by the end of treatment.
[b]Four of them recovered to grade 1 by the end of treatment.
CHF, congestive heart failure, NCI–CTC v2.0, National Cancer Institute—Common Toxicity Criteria version 2.0.
LLN, lower limit of normal.

discussion

This phase II trial shows that a regimen containing docetaxel (60 mg/m^2), LD (50 mg/m^2) and trastuzumab, with pegfilgrastim support, offers a 27% pCR rate in both breast and axilla. We would like to emphasize that almost half of the patients had grades 5 + 4 Miller and Payne pathological response. Several trials have shown good long-term outcomes in patients achieving grade 4 pathological response (residual T1a or minimal residual disease) [12–14]. Based on that, grades 4 + 5 Miller and Payne pathological response has been used as a main end point in other studies like the XENA trial with capecitabine, docetaxel and trastuzumab [15]. pCR assessment is far from being homogeneous when looking through the literature; in fact, almost 10 different pCR evaluation systems are used [16]. The most important discrepancy is the consideration of pCR in breast only, like the NSABP-B27 [17], or in both breast and axilla, like the trial of Buzdar et al. [18].

Another important efficacy measure of an NAC strategy in breast cancer is the rate of BCS. As a matter of fact, the possibility of BCS is one of the most important theoretic justifications for NAC. In our trial, the majority of patients could be treated with a BCS procedure. This is especially remarkable in this group of patients with a median tumor size of 3.4 cm and high proportion of stage IIIA patients (32%). However, longer follow-up is necessary to study the local relapse rate in this group of patients.

Focusing on regimens containing anthracycline and trastuzumab, our trial is one of the largest phase II studies published until now. Most published phase II clinical trials reported pCR rates <20%. Wenzel et al. [19] reported 7% pCR rate in 14 patients and Kelly et al. [20] reported 19% pCR rate in 52 patients. The most important trial in this setting is the

randomized one by Buzdar et al. [18]. This trial compared the addition of trastuzumab to sequential paclitaxel followed by 5-fluorouracil-epirubicin-cyclophosfamide versus the same regimen without trastuzumab. The trastuzumab arm resulted in an impressive 60% pCR rate, after the addition of a cohort of 22 non-randomized patients. The randomized part of the trial was stopped early after a non-planned analysis of only 42 patients. This decision raised a huge controversy about the early stopping rules in clinical trials [21].

We used an anthracycline plus trastuzumab regimen, with which we had initially a toxicity concern. However, except from non-febrile neutropenia, the other grades 3–4 toxic effects were rare, with <10% for all toxic effects. In fact, excluding emesis and fatigue, the yielded toxicity rates were <5%. These results are a reflection of a proper design of the previous phase I trial [1] with a correct choice of the recommended phase II dose.

The use of less cardiotoxic anthracyclines, instead of doxorubicin, is a particular good option, especially when associated with other cardiotoxic drugs such as trastuzumab. In fact, the worst cardiotoxicity grade, symptomatic CHF, is not rarely observed in trials exploring anthracyclines and trastuzumab in combination [20–23]. Moreover, significant cardiotoxicity was described with trastuzumab in trials using a non-anthracycline-containing NAC regimen. Cisplatin–docetaxel–trastuzumab was used in one trial [24] in which one case of CHF was observed and 55% of patients experienced grade 1 or 2 cardiotoxicity. Carboplatin–docetaxel–trastuzumab was used in another trial and 2 of 70 patients were prematurely withdrawn due to LVEF decline [25]. The use of epirubicin has resulted in mixed results. A phase II trial of epirubicin–docetaxel–trastuzumab [22] in metastatic breast cancer patients was prematurely stopped due to a high cardiotoxicity rate. On the contrary, in the aforementioned trial of Buzdar et al., the cardiotoxic profile was considered excellent by the authors, though 1 of 45 patients developed CHF. The results of the present study, conducted in 59 patients treated with a regimen containing anthracycline–trastuzumab, showed only asymptomatic cardiotoxicity. Therefore, we can state that the cardiotoxic profile observed in our study is remarkable. These results are in agreement with the previous studies of LD and trastuzumab combinations for locally advanced or metastatic breast cancer patients [26, 27]. In these trials, including heavily pretreated patients, also with anthracyclines, only asymptomatic decline of LVEF was observed without symptomatic CHF.

In conclusion, a regimen of LD 50 mg/m^2 and docetaxel 60 mg/m^2, every 21 days, with standard dose of trastuzumab and pegfilgrastim support offers a high response rate with a good cardiotoxicity profile, when used as an NAC for stages II and IIIA HER2-overexpressing breast cancer patients. Therefore, this regimen should be considered as a new option in the treatment of these patients.

funding

Cephalon Pharma; Roche Pharma.

acknowledgements

Authors thank María Isabel Casas (GEICAM) for her contribution in the statistical analysis of this study. Writing assistance was supported by Marta Mas from Trial Form Support. We thank also Dr A. Arcusa from the Consorci Sanitari de Terrasa, Dr A. Oltra from the Hospital Virgen de Los Lirios, Alicante, and Dr B. Cirauqui from the Hospital Universitari Germans Trias i Pujol, Badalona, Spain, for their valuable support in the inclusion of patients. Amgen Spain, Cephalon Pharma, Roche and Sanofy-Aventis provided the medication for the study.

disclosure

The authors declare there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications or opinions stated.

references

1. Kaufmann M, von Minckwitz G, Bear HD et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: new perspectives 2006. Ann Oncol 2007; 18: 1927–1934.
2. Fisher B, Gunduz N, Saffer EA. Influence of the interval between primary tumor removal and chemotherapy on kinetics and growth of metastases. Cancer Res 1983; 43: 1488–1492.
3. Fisher B, Bryant J, Wolmark N et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16: 2672–2685.
4. Kuerer HM, Newman LA, Smith TL et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol 1999; 17: 460–469.
5. 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; 26: 1231–1238.
6. Rahman AM, Yusuf SW, Ewer MS. Anthracycline-induced cardiotoxicity and the cardiac-sparing effect of liposomal formulation. Int J Nanomedicine 2007; 2: 567–583.
7. 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.
8. 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.
9. Batist G, Harris L, Azarnia 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.
10. Antón A, Ruiz A, Seguí MA et al. 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. Ann Oncol 2009; 20: 454–459.
11. 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.
12. Symanns WF, Peintinger F, Hatzis C et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007; 25: 4414–4422.
13. Peintinger F, Buzdar AU, Kuerer HM et al. Hormone receptor status and pathologic response of HER2-positive breast cancer treated with neoadjuvant chemotherapy and trastuzumab. Ann Oncol 2008; 19: 2020–2025.

14. Gralow JR, Burstein HJ, Wood W et al. Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease. J Clin Oncol 2008; 26: 814–819.
15. Glück S, McKenna EF Jr, Royce M. XeNA: capecitabine plus docetaxel, with or without trastuzumab, as preoperative therapy for early breast cancer. Int J Med Sci 2008; 5: 341–346.
16. Kuroi K, Toi M, Tsuda H et al. Issues in the assessment of the pathologic effect of primary systemic therapy for breast cancer. Breast Cancer 2006; 13: 38–48.
17. Bear HD, Anderson S, Smith RE et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2003; 21: 4165–4174.
18. 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.
19. Wenzel C, Hussian D, Bartsch R et al. Preoperative therapy with epidoxorubicin and docetaxel plus trastuzumab in patients with primary breast cancer: a pilot study. J Cancer Res Clin Oncol 2004; 130: 400–404.
20. Kelly H, Kimmick G, Dees EC et al. Response and cardiac toxicity of trastuzumab given in conjunction with weekly paclitaxel after doxorubicin/cyclophosphamide. Clin Breast Cancer 2006; 7: 237–243.
21. Puglisi F, de Azambuja E, de Castro G Jr, Demonty G. Shrinking the tumor, shrinking the patient sample size: the early disclosure dilemma. J Clin Oncol 2005; 23: 6803–6804.
22. 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.
23. 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.
24. Hurley J, Doliny P, Reis I et al. Docetaxel, cisplatin, and trastuzumab as primary systemic therapy for human epidermal growth factor receptor 2-positive locally advanced breast cancer. J Clin Oncol 2006; 24: 1831–1838.
25. Coudert BP, Largillier R, Arnould L et al. Multicenter phase II trial of neoadjuvant therapy with trastuzumab, docetaxel, and carboplatin for human epidermal growth factor receptor-2-overexpressing stage II or III breast cancer: results of the GETN(A)-1 trial. J Clin Oncol 2007; 25: 2678–2684.
26. Theodoulou M, Batist G, Campos S et al. Phase I study of nonpegylated liposomal doxorubicin plus trastuzumab in patients with HER2-positive breast cancer. Clin Breast Cancer 2009; 9: 101–107.
27. Cortes J, Di Cosimo S, Climent MA et al. Nonpegylated liposomal doxorubicin (TLC-D99), paclitaxel, and trastuzumab in HER2-overexpressing breast cancer: a multicenter phase I/II study. Clin Cancer Res 2009; 15: 307–314.