

## Nonpegylated Liposomal Doxorubicin (TLC-D99), Paclitaxel, and Trastuzumab in HER-2-Overexpressing Breast Cancer: A Multicenter Phase I/II Study

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**Abstract Purpose:** To determine the recommended dose, cardiac safety, and antitumor activity of nonpegylated liposomal doxorubicin (TLC-D99), paclitaxel, and the anti-HER-2 monoclonal antibody trastuzumab in patients with HER-2-overexpressing locally advanced nonoperable breast cancer (LABC) and metastatic breast cancer (MBC).  
**Experimental Design:** Women with measurable, previously untreated, HER-2-overexpressing LABC and MBC with a baseline left ventricular ejection fraction (LVEF)  $>50\%$  received weekly trastuzumab in combination with escalating doses of weekly paclitaxel and TLC-D99 every 3 weeks for 6 cycles. LVEF monitoring was done every 3 weeks for the first 18 weeks and every 8 weeks thereafter.  
**Results:** Sixty-nine patients participated, 15 in the dose escalating part and 54 at the recommended phase II dose (28 patients with LABC and 26 patients with MBC). The recommended doses of TLC-D99 and paclitaxel were  $50 \text{ mg/m}^2$  every 3 weeks and  $80 \text{ mg/m}^2/\text{wk}$ , respectively. Twelve (17%) patients developed asymptomatic declines in LVEF. In 8 of these patients, LVEF recovered to  $\geq 50\%$  after a median time of 9 weeks (range, 3-38 weeks). In the rest of patients, LVEF ranged from 44% to 49%. No patients developed symptomatic cardiac heart failure. The overall response rate was 98.1% (95% confidence interval, 90.1-99.9) with a median time to progression not reached in LABC and of 22.1 months (95% confidence interval, 16.4-46.3) in MBC patients.  
**Conclusions:** Nonpegylated doxorubicin, paclitaxel, and trastuzumab combination is safe, does not result in clinically manifest cardiac toxicity, and has a high rate of durable responses in HER-2-overexpressing breast cancer patients. Further exploration of this combination is warranted.

Overexpression/amplification of the HER-2 is found in ~20% to 30% of invasive breast cancer and correlates with worse clinical outcome (1, 2). Trastuzumab, a humanized monoclonal antibody targeting HER-2 (3), is clinically active in HER-2-

overexpressing advanced breast cancer patients (4-7) and improves survival when combined with taxanes (8, 9). Based on these studies, the combination of trastuzumab with taxanes is currently considered a standard of care for patients with HER-2-overexpressing advanced breast cancer.

In the pivotal phase III study that led to the approval of trastuzumab (8), one of the treatment arms evaluated the combination of doxorubicin-cyclophosphamide and trastuzumab. This arm showed an unexpected high rate of cardiac toxicity with 16% of women experiencing symptomatic congestive heart failure (CHF), which led to the recommendation of not administering trastuzumab with anthracyclines. However, the doxorubicin-cyclophosphamide with trastuzumab arm resulted in a statistically significant improvement of clinical efficacy over doxorubicin-cyclophosphamide alone, including a gain in overall survival (8).

Despite the cardiac toxicity observed, several considerations prompted us and others to continue to explore the combination of anthracyclines and trastuzumab. First, in preclinical models, this combination was highly effective (10) and the clinical benefit observed in the pivotal phase III trial was greater with trastuzumab combined with doxorubicin-cyclophosphamide than with paclitaxel (8). Second, the trastuzumab-related cardiac toxicity had not been anticipated in the early studies;

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### Translational Relevance

The study provides a new insight in the ongoing discussion of the use of anthracyclines and trastuzumab in patients with HER-2-overexpressing tumors. It has been well documented that HER-2-overexpressing tumors display enhanced sensitivity to anthracyclines and that anthracyclines and trastuzumab-containing combinations are highly active in patients with advanced breast cancer. The major concern with this approach has been the risk of enhanced cardiac toxicity. Our study shows that adding the nonpegylated liposomal doxorubicin (TLC-D99) to the approved trastuzumab plus paclitaxel regimen is feasible, is safe, and results in a high clinical benefit rate in patients with HER-2-overexpressing advanced breast cancer. In terms of cardiac safety, there were no cases of treatment-related clinical heart failure and we observed asymptomatic decreases in LVEF that recovered to levels  $\geq 50\%$  in the majority of patients. The combination was highly active with an ORR of 98.1%, among the highest reported in HER-2-overexpressing breast cancer patients. In summary, the use of a nonpegylated liposomal anthracycline may provide the important benefit of anthracyclines and trastuzumab while diminishing the risk of cardiac toxicity. As a result of our study, this concept is now being tested in a large randomized phase III study.

therefore, patient selection and cardiac monitoring were not optimal. For example, a subsequent review of these early clinical trials data found that the incidence of symptomatic cardiac events was greatest in patients previously treated with anthracyclines (11). Third, newer series of clinical trials combining anthracyclines and trastuzumab, with a limited amount of anthracyclines administered and more restrictive entry and monitoring criteria, have shown these combinations to be safe and among the most active in the advanced and early disease setting (12–14). We studied the administration of doxorubicin, paclitaxel, and trastuzumab in 16 patients with metastatic HER-2-overexpressing breast cancer (12). The combination was highly active with a response rate of 88% and did not result in clinical cardiac toxicity, and decreases in the left ventricular ejection fraction (LVEF) were minimal and reversible (12). Similarly, a phase I trial with epirubicin, cyclophosphamide, and trastuzumab had response rates up to 60%, and cardiac toxicity was limited to asymptomatic LVEF decreases (13). In early disease, the randomized phase III neoadjuvant NOAH study has compared a doxorubicin and paclitaxel-containing regimen with and without trastuzumab. Of 115 patients treated with doxorubicin, paclitaxel, and trastuzumab, only 11% had asymptomatic decreases of LVEF  $\geq 10\%$  with no cases of clinical CHF (14). This arm resulted in a statistically significant improvement in outcome with a pathologic complete response rate of 43% (14).

An additional approach to minimize the cardiac toxicity of anthracyclines and trastuzumab is to use the novel formulations of liposomal anthracyclines (15, 16). One such formulation is the nonpegylated liposome doxorubicin TLC-D99 (Myocet; Zeneus Pharma). Two randomized trials comparing TLC-D99 versus conventional doxorubicin either as single agent or in combination with cyclophosphamide as first-line treat-

ment for metastatic breast cancer (MBC) showed equivalent antitumor efficacy and a statistically significant reduction in cardiac toxicity (16, 17).

Based on the promising results of anthracycline, taxanes, and trastuzumab in the above-mentioned pilot studies and the enhanced cardiac safety profile of TLC-D99, we decided to evaluate the safety and efficacy of the novel combination TLC-D99, paclitaxel, and trastuzumab as first-line treatment in patients with HER-2-overexpressing advanced breast cancer.

### Patients and Methods

**Patient selection.** Eligible patients were ages 18 to 70 years with HER-2-overexpressing, locally advanced nonoperable breast cancer (LABC) or MBC. HER-2 overexpression was assessed at each institution at study entry by immunohistochemistry (3+, Herceptest; DAKO) or a positive fluorescence *in situ* hybridization for *HER-2* amplification (PathVision; Vysis). Patients had at least one bidimensionally measurable lesion according to WHO criteria (18); an Eastern Cooperative Oncology Group performance status of 0 or 1; and adequate cardiac function, with LVEF  $>50\%$  at baseline and no history of myocardial infarction. Patients with bone, as unique disease localization, and central nervous system metastases were excluded from the study. Patients with MBC were allowed prior adjuvant or neoadjuvant chemotherapy if completed at least 4 weeks before enrolment, but prior administration of anthracyclines, taxanes, or trastuzumab was not permitted. Ethics committee from participating center approved the study, and all patients gave prior written informed consent. Trial data were monitored according to Good Clinical Practice guidelines.

**Study design and objectives.** The study was designed to be an open-label, multicenter, phase I/II clinical trial using a two-stage optimal design. The aim of the phase I dose escalating part of the study was to determine the recommended dose for the phase II expansion part of the study. The primary aims of the phase II part of the study were to evaluate the safety and efficacy of the combination at the recommended doses. Overall response rate (ORR) was defined as the total of complete response (CR) plus partial response. Secondary endpoints included time to progression (TTP), time to response, duration of response, and overall survival.

**Treatment plan.** Study treatment consisted of nonpegylated liposome doxorubicin (TLC-D99), 1 h intravenous infusion, day 1, every 3 weeks, for 6 cycles; paclitaxel, 1 h intravenous infusion, day 1, weekly, for up to 52 weeks; and trastuzumab, loading dose of 4 mg/kg intravenous infusion over 90 min, day 1, and 2 mg/kg intravenous infusion over 30 min, weekly thereafter for up to 52 weeks. No concurrent endocrine treatment was allowed.

In the dose escalation part of the study, patients were treated in cohorts of escalating doses of TLC-D99 and paclitaxel. Treatment-related adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. Dose-limiting toxicity (DLT) was defined as grade 4 neutropenia persisting for  $>5$  days, febrile neutropenia, other grade 4 hematologic toxicity or any complicated grade 3 hematologic toxicity, or any grade 3/4 nonhematologic toxicity apart from nausea, vomiting, and alopecia. Three patients were enrolled per dose level. If one case of DLT was observed, the cohort was expanded to include 6 patients. The Maximum tolerated dose was defined as the dose level at which  $\leq 33\%$  of the patients would experience DLT.

The expansion part of the study was to be conducted at the recommended dose (the dose level immediately below the maximum tolerated dose) or at dose level 5 if the maximum tolerated dose was not reached.

At the end of treatment with TLC-D99, patients with LABC could undergo surgery if indicated. Treatment with trastuzumab and paclitaxel was resumed 4 weeks after surgery up to the completion of 52 weeks of treatment.

**Table 1.** Patient characteristics

	Total (n = 69)	LABC (n = 32)	MBC (n = 37)
Median (range) age, y	53 (26-78)	55 (26-78)	53 (30-74)
Hormone receptor status, n			
ER+ and PgR+	20	7	13
ER+ and PgR-	9	7	2
ER- and PgR-	34	14	20
Unknown	6	4	2
Eastern Cooperative Oncology Group performance status, n			
0	50	29	21
1	19	3	16
2	0	0	0
Sites of disease, n			
Lymph node	43	20	23
Bone	17	0	17
Liver	18	0	18
Lung	16	0	16
Skin	4	0	4
Pleural effusion	5	0	5
Ascites	1	0	1
Ovary	2	0	2
Prior cancer therapy, n			
Adjuvant chemotherapy	9	0	9
Adjuvant radiotherapy	10	0	10
Hormonal therapy	8		8

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

At the end of study treatment period, 6 cycles of TLC-D99 plus paclitaxel and trastuzumab for 52 weeks, patients with stable or responding disease could continue with trastuzumab alone (either weekly or every 3 weeks; ref. 7) at the investigator's criteria until disease progression.

**Cardiac monitoring.** Cardiac function was monitored by 12-lead electrocardiogram and LVEF measurement by echocardiography or multiple-gated acquisition scan every 3 weeks for the first 18 weeks; thereafter, electrocardiograms were done every 4 weeks and LVEF measured every 8 weeks until month 18 from treatment start. A cardiac event was defined as type A in cases with clinically manifest CHF that included a S3 gallop, tachycardia, dyspnea, orthopnea, and/or edema consistent with CHF plus a grade 3/4 LVEF decline. A cardiac event was defined as type B in case of asymptomatic LVEF decline: an absolute drop  $\geq 10\%$  resulting in a final LVEF  $< 50\%$ , LVEF  $< 40\%$ , or any absolute drop  $\geq 20\%$ .

If a type A cardiac event occurred, the patient was to be withdrawn from the study and accrual was stopped, although treatment would continue as planned in the other enrolled patients. If a type B cardiac event occurred, TLC-D99 was discontinued, but the patient could continue with paclitaxel and trastuzumab; if the LVEF did not return to baseline levels within the next two consecutive cardiac assessments, then the patient was withdrawn from study. Safety analysis included all patients receiving at least one dose of the study treatment.

**Response assessment.** Tumor response was assessed according to standard WHO criteria (18) at weeks 6, 12, and 18 and every 8 weeks thereafter. The rate of pathologic CR (pCR), defined by the absence of invasive carcinoma in the breast and in the axillary nodes, was also calculated in patients who underwent surgery.

TTP was calculated from treatment initiation to disease progression or death from any cause. Time to response was calculated from treatment initiation to the date of objective response. Duration of response was calculated from the date of objective response to the date of progression. Overall survival was defined from treatment initiation to the date of death or last date the patient was known to be alive. Efficacy analysis included all patients in the expansion part of the study who received at least one dose of study treatment.

**Statistical methods.** A Simon's optimal two-stage design was applied. The sample size was estimated to detect a 70% response rate, with 60% for a minimal hypothesis. In the first stage, 24 responses of 40 patients were required. With a type I error and a type II error of 0.20, a total of 69 patients were planned with at least 44 responses required overall. Accrual was planned to stop as soon as the number of required responses by the statistical design was achieved. The Kaplan-Meier method was used to estimate time to event distributions. The analysis was done with SAS version 9.1.3 (SAS Institute). Results.

Between December 2000 and September 2003, a total of 69 patients were enrolled across study sites in Spain. Patient's characteristics are summarized in Table 1. Noteworthy, the unique case of inflammatory breast cancer presented with metastases at the time of initial diagnosis (stage IV).

**Phase I.** A total of 21 patients were treated with escalating doses. Dose escalation proceeded as planned up to including the highest dose level. No DLT occurred in the initial 3 patients treated at dose levels 1 to 4 (Table 2); 2 of the 6 patients treated at dose level 5 experienced a DLT (febrile neutropenia). Dose level 4 was therefore expanded from the initial 3 patients to include a total of 6 patients, without DLT being identified. Dose level 4 [TLC-D99 50 mg/m<sup>2</sup> every 3 weeks, paclitaxel 80 mg/m<sup>2</sup>/wk, and trastuzumab 2 mg/kg/wk (after a loading dose of 4 mg/kg)] was therefore chosen to be further evaluated in phase II expansion.

**Phase II.** Forty-eight additional patients were enrolled at the recommended phase II dose for a total of 54 patients in this part of

**Table 2.** Dose levels and number of patients entered

Dose level	No. patients	Dose			DLTs*
		Nonpegylated liposomal doxorubicin (mg/m <sup>2</sup> /3 wk)	Paclitaxel (mg/m <sup>2</sup> /wk)	Trastuzumab (mg/kg/wk) <sup>†</sup>	
1	3	40	60	2	0
2	3	40	70	2	0
3	3	50	70	2	0
4	Phase I: 6 (expansion: 48) <sup>‡</sup>	50	80	2	0
5	6	60	80	2	2
Total	21				

\*DLT: 1 patient grade 3 and 1 patient grade 4 febrile neutropenia.

<sup>†</sup>After an initial loading dose of 4 mg/kg.

<sup>‡</sup>Phase II included the original 6 patients from dose level 4 in phase I plus an additional 48 patients.

the study. Patient compliance with study treatment was good. Thirty-nine (72%) patients completed 6 cycles of TLC-D99 plus 18 weeks of paclitaxel and trastuzumab, 26 (48%) patients completed at least half of the planned weeks of treatment with paclitaxel and trastuzumab, and 17 patients (32%) completed all the 52 weeks of the study treatment.

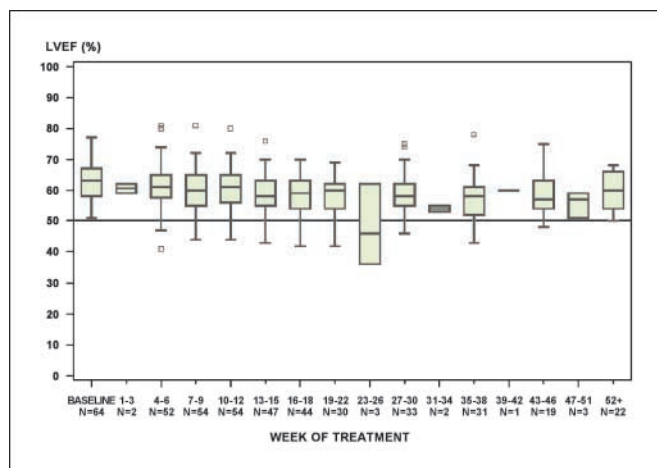
**Changes in cardiac function.** Overall, the LVEF values were maintained within the normal range throughout the study treatment period. The median LVEF at baseline was 63.0% [95% confidence interval (95% CI), 61.4-64.6], declining to 60% (95% CI, 56.3-61) after 6 cycles of TLC-D99 and to 59% (95% CI, 55.6-61) after 52 weeks of paclitaxel and trastuzumab (Fig. 1).

No patient developed treatment-related symptomatic CHF. A total of 12 patients (1 from the third level of phase I and 11 from phase II) experienced asymptomatic protocol-defined cardiotoxicity, that is, an absolute decline in LVEF  $\geq 10\%$  resulting in final LVEF  $< 50\%$ , LVEF  $< 40\%$ , or any absolute decline in LVEF  $\geq 20\%$  (Table 3A). The median decline in LVEF from baseline was 14% (range, 6.5-23%) and the median time to LVEF decline was 16 weeks (range, 4-43 weeks). LVEF recovered to  $\geq 50\%$  in 8 of the patients after a median time of 9 weeks (range, 3-38 weeks). In 3 additional patients, the LVEF recovered to  $> 45\%$ .

Two CHF events were reported: 1 patient suffered a bacterial endocarditis secondary to a methicillin-resistant *Staphylococcus aureus* infection soon after the insertion of a port-a-cath venous access device on week 5. The LVEF recovered to 65% after 14 days. A second patient experienced CHF in the context of febrile neutropenia, anemia, and hypotension on week 12. The LVEF recovered to 55% after 15 days. None of the patients experienced additional episodes of cardiac toxicity during study treatment period and follow-up. Considering the time to recovery of LVEF and the context of systemic infection, both CHF events were not considered treatment-related.

**Adverse events.** Overall, the most common adverse events included alopecia (85.1%), mucosal inflammation (57.4%), nausea (50%), and asthenia (51.8%). Treatment-related adverse events occurring in  $> 10\%$  of patients are reported in Table 3B.

Eleven (20.3%) patients were withdrawn from the study treatment due to adverse events, including neutropenia and febrile neutropenia (7.4%), neuropathy (1.8%), bilateral pneumonia and neutropenia (1.8%), dermatitis (3.7%), decrease in performance status (1.8%), and pancreatitis (1.8%). Eight (15%) additional patients were withdrawn from treatment due to asymptomatic protocol-defined cardiac adverse events. No treatment-related deaths occurred.



**Fig. 1.** LVEF over time. Whiskers (standard span) were extended to 1.5 times the interquartile range outside of the first and third quartiles. *Square*, outliers beyond the standard span.

**Efficacy.** All 54 patients in the recommended phase II dose were assessable for tumor response; 29 patients achieved a CR (53.7%) and 24 patients a partial response (44.4%) for an ORR of 98.1% (95% CI, 90.1-99.9). All patients with LABC responded and 16 of 28 of these patients with initially unresectable disease underwent surgery. Two of these patients achieved a pCR. In patients with MBC, 25 of 26 achieved a response; the remaining patient had stable disease (Table 4).

Median time to response was 1.87 months (95% CI, 1.84-2.00) in LABC and 1.87 months (95% CI, 1.84-1.94) in MBC patients for an overall median time to response of 1.87 months (95% CI, 1.84-2.00).

As of July 2007, 36 patients were alive. Median duration of response has not been yet reached in LABC patients and was of 19.3 months (95% CI, 14.4-44.9) in MBC patients for an overall median duration of response of 38.6 months (95% CI, 18.2-not reached). Median TTP has not been reached in LABC patients and was of 22.1 months (95% CI, 16.4-46.3) in MBC patients for an overall median TTP of 40.8 months (95% CI, 21.2-not reached; Fig. 2). Median overall survival was 61.4 months (95% CI, 57.9-not reached) and 40.4 months (95% CI, 20.4-not reached) in LABC and MBC patients, respectively.

## Discussion

This study shows that adding the nonpegylated liposomal doxorubicin (TLC-D99) to the approved trastuzumab plus paclitaxel regimen is feasible and safe and results in a high clinical benefit rate in patients with HER-2-overexpressing advanced breast cancer. The combination was well tolerated, with the observed toxicities in the range of those reported with trastuzumab plus taxanes in similar patient population (8, 9, 19, 20). Cardiac function was closely monitored, no case of treatment-related clinical heart failure was reported, and asymptomatic declines in LVEF were minimal and reversible. The combination was highly active with an ORR of 98.1% among the highest reported in HER-2-overexpressing breast cancer patients.

Our safety results are in line with those of a parallel study of TLC-D99 and trastuzumab in patients with advanced HER-2-overexpressing advanced breast cancer (21). In this phase II study, 37 patients were treated with TLC-D99 60 mg/m<sup>2</sup> and trastuzumab (21) for an ORR of 59% (95% CI, 41-75). Two cardiac events were reported: 1 asymptomatic, in a patient previously treated with doxorubicin and trastuzumab, and 1 symptomatic, in a patient receiving doxorubicin 6 weeks before entering the study. Both patients recovered off study. The mechanism by which TLC-D99 has a reduced cardiac toxicity may be due to a lower myocardium exposure to anthracycline (22, 23). In support of this, the mean clearance of total doxorubicin after TLC-D99 administration is ~5-fold lower and the volume of distribution is 10-fold lower than with conventional doxorubicin (24). Furthermore, both peak and overall concentrations of doxorubicin in myocardial tissue are reduced by 30% to 40% on TLC-D99 compared with doxorubicin (23). In our study, the recovery of LVEF in patients withdrawn from the study is more likely to be the result of a reversible myofibrillar dysfunction that occurs with trastuzumab rather than the cell death injury that results from the typical anthracycline injury, a rarely reversible event (25-28). Of note, 5 of 69 (7%) patients presented persistent asymptomatic decline of LVEF  $< 50\%$ . Although the clinical significance of LVEF decline on treatment with trastuzumab is currently being studied in the adjuvant setting, it appears a reasonable risk for

**Table 3.****(A) Cardiac safety profile: asymptomatic LVEF declines: patient characteristics, timing of onset, and recovery**

Age	Cardiac risk factors	Baseline LVEF (%)	Registered LVEF (%)	Absolute decline (%)	Timing of decline (week of treatment)	Recovery to LVEF $\geq$ 50% (wk)	Discontinuation of study	Final LVEF (last monitoring; %)
54	None	56	49.5	6.5	16	5	No	50 (month 18)
			42	14	25	32	Yes	
64	Hyperlipidemia	56	43	13	16	7	No	62 (month 12)
42	None	59	43	16	19	Not recovered	Yes	45 (month 18)
48	None	53	44	9	10	38	Yes	57 (month 18)
56	None	57	43	14	35	18	Yes	61 (month 15)
61	None	62	49	13	51	22	Yes	50 (month 18)
31	None	58	46	12	31	9	Yes	56 (month 15)
66*	Angina pectoris	58	47	11	4	3	No	49 (month 15)
39	None	66	43	23	13	Not recovered	Yes	49 (month 18)
37	None	55	36	19	35	Not recovered	Yes	47 (month 18)
62	Hypertension	64	47	17	16	19	No	51 (month 12)
	Hyperlipidemia		49	15	43	9	No	
44	None	66	46	20	16	Not recovered	Yes	44 (month 18)

**(B) Hematologic and nonhematologic adverse events<sup>†</sup>**

Event	Any grade, n (%)	Grade 3/4, n (%)
Hematologic		
Neutropenia	16 (29.6)	12 (22.27)
Febrile neutropenia	—	11 (20.4)
Anemia	7 (12.9)	3 (5.56)
Nonhematologic		
Alopecia <sup>46</sup> (85.19)	9 (16.67)	
Mucosal inflammation	31 (57.41)	4 (7.41)
Nausea	27 (50)	4 (7.4)
Asthenia	28 (51.85)	3 (5.56)
Vomiting	21 (38.89)	2 (3.7)
Nail disorder	17 (31.48)	—
Diarrhea	26 (48.15)	4 (7.41)
Paresthesia	14 (25.93)	1 (1.85)
Fatigue	13 (24.07)	2 (3.7)
Stomatitis	7 (12.96)	2 (3.7)
Onicolysis	8 (14.81)	1 (1.85)
Pyrexia	17 (31.48)	
Erythema	13 (24.07)	
Dyspepsia	8 (14.8)	
Anorexia	6 (11.1)	
Conjunctivitis	7 (12.96)	
Epistaxis	7 (12.96)	
Myalgia	6 (11.1)	
Upper abdominal pain	7 (12.96)	
Skin hyperpigmentation	7 (12.96)	

\*From phase I, level 3.

<sup>†</sup>National Cancer Institute Common Toxicity Criteria version 2.0.

an improved outcome in patients with a poor prognosis such as those with advanced breast cancer.

The high ORR and prolonged TTP in our study are similar with those previously reported with the combination of doxorubicin, paclitaxel, and trastuzumab in the advanced disease setting (12). Despite the observed high response rate, the pCR rate in patients with locally advanced disease was relatively modest (7%). The most likely explanation for this low pCR rate is that our study population had LABC. In support of this hypothesis, other neoadjuvant studies with the combination of anthracyclines, paclitaxel, and trastuzumab that have

included patients with less advanced disease have shown higher pCR rates. The neoadjuvant phase III NOAH study in patients with HER-2-overexpressing early breast cancer has reported a 43% pCR rate with an anthracycline, paclitaxel, and trastuzumab regimen (14), and a pilot neoadjuvant study of trastuzumab combined with paclitaxel followed by FEC observed a pCR rate of 65% (29).

Another point for discussion is whether our results are in line with those observed with other liposomal doxorubicin formulations. In addition of the nonpegylated formulation studied here, there is also commercially available a pegylated

**Table 4.** Rates and duration of responses

	Total of patients	LABC	MBC
All patients, <i>n</i>	54	28	26
Objective response, <i>n</i> (%)	53 (98.1)	28 (100)	25 (96.2)
CR, <i>n</i> (%)	29 (53.7)	20 (71.4)	9 (34.6)
Partial response, <i>n</i> (%)	24 (44.4)	8 (28.6)	16 (61.5)
Median (95% CI) time to response, mo	1.87 (1.84-1.94)	1.87 (1.84-2.00)	1.87 (1.84-1.94)
Median (95% CI) duration of response, mo	38.6 (18.2-NR)	NR	19.3 (14.4-44.9)

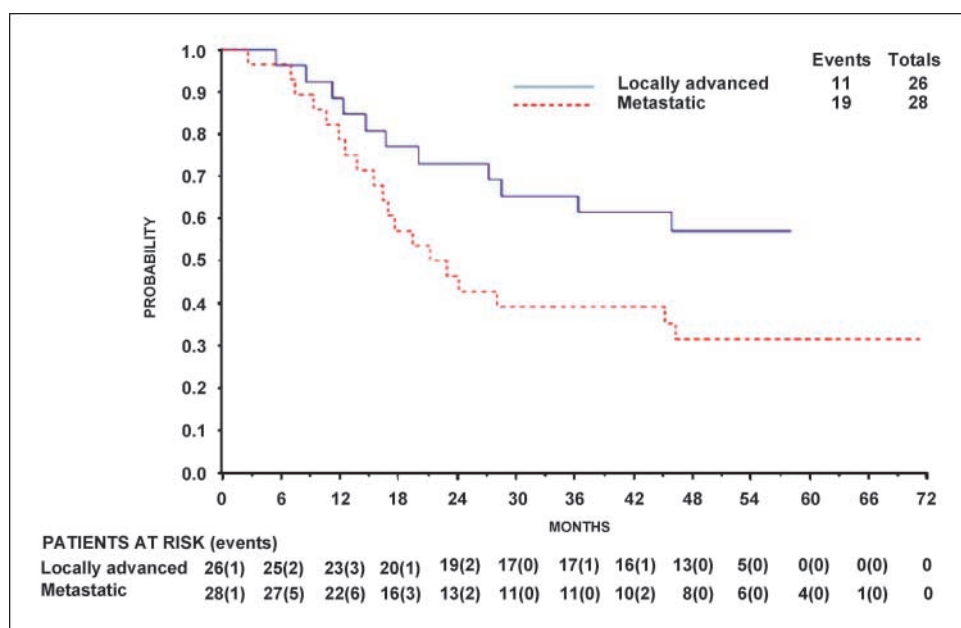
Abbreviation: NR, not reached.

liposomal doxorubicin formulation. Pegylated and nonpegylated doxorubicin have a different safety profile. Whereas nonpegylated toxicities clearly resemble those of doxorubicin, the toxicities of pegylated doxorubicin include hand-foot syndrome, stomatitis, and mucositis (15–17). This is important as it may limit the possibility of combinatorial approaches. In a small study, treatment with pegylated liposomal doxorubicin and trastuzumab resulted in stable disease in 8 of 12 heavily pretreated breast cancer patients (30). In another study, the response rate was 52% with an additional 38% stable disease when the same treatment was offered as front-line therapy to 30 MBC patients (31). Cardiac toxicity was limited to asymptomatic declines of LVEF in both refractory and first-line setting (30, 31). However, due to an unexpected 40% incidence of grade 3 hand-foot syndrome, the same treatment in combination with docetaxel was considered not feasible (32). Hence, it is likely that based on its toxicity profile TLC-99 may be better suited to be given in combination with taxanes.

The results of our study may compare favorably with other highly active regimens in patients with HER-2-overexpressing advanced breast cancer. For example, triple-agent first-line regimens including trastuzumab/docetaxel/platinum salts (33),

trastuzumab/paclitaxel/platinum salts (34, 35), trastuzumab/paclitaxel/gemcitabine (36), and trastuzumab/docetaxel/capecitabine (37) achieve ORRs ranging from 50% to 80%.

A potential explanation for the high activity rate in our study is that HER-2-overexpressing tumors may have enhanced sensitivity to anthracyclines (38–40), a clinical observation that been mechanistically linked to a higher expression of topoisomerase II  $\alpha$  in HER-2-amplified tumors. The *topoisomerase II $\alpha$*  gene is located adjacent to the HER-2 gene at the chromosome location 17q12-q21 and is coamplified with HER-2 in ~30% to 40% of primary HER-2-overexpressing breast cancer (41). An important discussion point is the future role of anthracyclines in the therapy of early HER-2-overexpressing breast cancer. The NOAH neoadjuvant study and the overall safety of the trastuzumab anthracycline-containing adjuvant studies (42–44) suggest that anthracyclines will continue to be an important component in the treatment of early disease. In this setting, TLC-D99 could enhance the cardiac safety of these regimens without compromising their clinical activity. On the other hand, there will be patients that will not receive anthracycline-containing regimens in the adjuvant setting. For example, one of the arms of the BCIRG trastuzumab adjuvant



**Fig. 2.** TTP curves of LABC and MBC patients entered in phase II study (*n* = 54).

study contained a taxane and platinum combination (45). Consequently, an increasing number of patients not previously exposed to anthracyclines could, on relapse, benefit from TLC-D99 and trastuzumab-based combinations.

In summary, the results of our study support further exploration of this combination and we have launched a large, phase III trial in patients with advanced HER-2-overexpressing breast cancer in the first-line setting comparing TLC-D99, paclitaxel, and trastuzumab with paclitaxel and trastuzumab, and progression-free survival and cardiac safety are the primary and safety endpoints.

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