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ORIGINAL ARTICLE

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Pegylated liposomal doxorubicin in combination with gemcitabine: a phase II study in anthracycline-naïve and anthracycline pretreated metastatic breast cancer patients

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Abstract *Background*: The aim of the study was to assess the toxicity profile, activity in terms of response rate, time to progression, overall survival, and quality of life of pegylated liposomal doxorubicin (PLD) and gemcitabine combination in chemo-naïve and pretreated metastatic breast cancer (MBC) women. *Methods*: Patients were eligible if they had disease progression to prior chemotherapy (anthracycline-including or not) for early breast cancer or MBC. Patients received PLD 25 mg/m^2 intravenously on day 1 plus gemcitabine 800 mg/m^2 intravenously on days 1 and 8 of each 21-day cycle. *Results*: Of 50 patients enrolled, 37 had received prior adjuvant chemotherapy (24 with an anthracycline) and 23 prior chemotherapy for metastatic disease (6 with an anthracycline). Two complete responses and 20 partial responses were achieved in 46 assessable patients (overall response rate: 47.8%). Responses were observed in 14 (46.6%) of 30 patients with previous anthracycline exposure. Median response duration was 7 months, median duration of clinical benefit 8 months, time to progression 7 months. At a median follow-up of 10 months, 79.4% patients were alive at 1 year. No neutropenic complication was observed. Non-hematological toxicities were mild. One patient previously treated with an anthracycline developed a transient decrease (26%) in the left ventricular ejection fraction, with cardiac function recovering within 6 months. *Conclusion*: Because of the non-overlapping toxicity profiles of both PLD and gemcitabine, this combination can be regarded as a reliable therapeutic option for patients who have failed previous treatments, including anthracycline, for MBC.

Keywords Liposomal doxorubicin · Gemcitabine · Metastatic breast cancer

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Introduction

The drugs usually given to chemo-naïve metastatic breast cancer (MBC) patients achieve response rates of 20–50% with a median response duration of 3–9 months when administered as single agents. Among these compounds, anthracyclines remain the most active drugs in the treatment of advanced breast cancer. While doxorubicin displays an excellent antitumor activity profile, its use in clinical practice is limited by drug-associated toxicities, particularly myelosuppression and cardiotoxicity.

A novel approach to improve the therapeutic index of anthracyclines is the development of analogs encapsulated in liposomes. Pegylated liposomes resulted in prolonged drug circulation time and better drug distribution in tumor tissue [1]. These compounds represent a unique formulation in which a polyethylene glycol layer surrounds the doxorubicin-containing liposome as a result of a process termed "pegylation", which protects

the liposomes from detection by the mononuclear phagocyte system and increases the plasma half-life compared with conventional doxorubicin [2, 3]. The size of the drug-containing vesicles allows the liposomes to extravasate through leaky tumor vasculature. This property, combined with its longer half-life, promotes targeted drug delivery to the tumor site [4]. Previous studies with pegylated liposomal doxorubicin (PLD) have initially demonstrated a comparable efficacy to classic doxorubicin in untreated advanced breast cancer patients [5] with a significant reduction of cardiotoxicity, myelosuppression, vomiting and alopecia and a remarkable improvement in quality of life [6].

Gemcitabine is an analog of deossicitidine that is enclosed into the nucleic acids instead of natural nucleoside; its structure is similar to the cytosine arabinoside molecule. Gemcitabine has now undergone considerable testing for various malignancies and has exhibited good activity and tolerability in non-small cell lung cancer, pancreatic cancer, bladder cancer, cisplatin-refractory ovarian carcinoma and advanced breast cancer. Multiple studies have established the activity of gemcitabine as a single agent in patients with MBC. Its activity has been mainly established as resistant to both anthracyclines and taxanes and higher responses rates (37–42%) have been registered in less heavily pretreated patients. The toxicities observed were typically mild and included neutropenia, thrombocytopenia, fatigue and anemia [7–10].

Combinations of PLD with several other chemotherapeutic agents are effective and tolerable in patients with MBC [11]. More importantly, combinations containing PLD are effective in patients with prior anthracycline exposure. This is particularly relevant, since many patients receive conventional doxorubicin in the adjuvant or metastatic setting. Considering the different mechanisms of action and non-overlapping toxicity profiles of PLD and gemcitabine as single agents, we combined these two agents in patients with MBC with the aim to develop a clinically effective combination regimen with improved efficacy, more convenient dosing schedules, better tolerability and fewer side effects. The results of a recent phase I study identified the maximum-tolerated dose of PLD as 24 mg/m^2 day 1 plus gemcitabine 800 mg/m^2 days 1 and 8 every 3 weeks [12]; subsequently a phase II study in non-pretreated MBC patients reported a good safety and activity [13].

Herein we report a further experience in a population of either naïve or pre-treated MBC women. The aim was to assess the toxicity profile, activity in terms of response rate, time to progression, overall survival and finally the quality of life of the combination of PLD with gemcitabine.

Patients and methods

Patients were required to have ≥18 years of age and cytological or histological diagnosis of metastatic breast carcinoma. They could have received prior anthracycline-including chemotherapy regimen for early breast cancer or MBC with a cumulative dose of doxorubicin ≤ 350 mg/m^2 or epirubicin ≤ 480 mg/m^2. Patients who did not receive an anthracycline-containing regimen (i.e, due to age, patient's refusal) entered the study as well. Patients had not previously received chemotherapy including liposomal doxorubicin and gemcitabine. Prior hormonal therapy was admitted. A bidimensionally measurable disease, adequate general health status and absence of active concomitant illnesses, such as uncontrolled infections, diabetes, severe cardiovascular or pulmonary disease, were required. Patients had a Performance Status (ECOG) ≤ 2 and a life expectancy ≥3 months. Patients with previous controlled neoplastic disease diagnosed over 5 years were allowed to enter the study. They were neither pregnant nor nursing. Patients with symptomatic central nervous system metastases were excluded. They were required to have a left ventricular ejection fraction (LVEF) of 50% or greater, absolute neutrophil count more than 1,500 per μl, platelet count more than 100,000 per μl, bilirubin less than 2 mg/dl, AST/ALT less than 3×upper normal limit (UNL). Other concurrent antineoplastic therapy was not admitted and patients had concluded any radiotherapy or chemotherapy at least 4 weeks and/or hormonal therapy at least 1 month before treatment. Patients could receive concomitant bisphosphonate treatment. The local Scientific and Ethical Committee approved the protocol and all women gave written informed consent before entering the study according to the Declaration of Helsinki.

Treatment plan. The treatment was given in an outpatient setting. Cycles consisted of PLD 25 mg/m^2, diluted in 5% dextrose 250 ml as intravenous administration over 60 min on day 1, and subsequently gemcitabine 800 mg/m^2 diluited in 0.9 saline solution 250 mL as intravenous route over 30 min on days 1 and 8, repeated every 3 weeks. Chemotherapy was administered until documented disease progression, unacceptable toxicity, patient's refusal or investigator's discretion. Routine prophylactic antiemetic support was given.

Patient monitoring. Patients were followed with complete blood cell count with differential on days 1, 8 and every 3 weeks while biochemical profile was repeated before each cycle of treatment. Two drug doses were adjusted during each course on the day of therapy based on hematological and non-hematological toxicity. Adjustments in the dose of PLD were made in patients who experienced grade 4 febrile neutropenia, grade 4 neutropenia persisting for 7 days, grade 4 thrombocytopenia or in case of grade ≥3 non-hematological toxicity. On day 8, a 25% dose reduction of gemcitabine was applied in the case of grade 2 neutropenia and/or thrombocytopenia and/or grade 2 liver toxicity: the reduction was 50% in case of grade 3 neutropenia; the drug was omitted in case of grade 4 neutropenia, febrile neutropenia, grade 3/4 thrombocytopenia or every grade

3/4 non-hematological toxicity, except for emesis. Granulocyte-colony stimulating factor (G-CSF) was used in case of treatment delay of more than 2 weeks because of neutropenia or febrile neutropenia. Hematological support with recombinant human erythropoietin was applied at the investigator's discretion. If serious toxicity persisted for more than 3 weeks despite dose adjustments or omissions, the patient was taken off the study.

Cardiac assessment with echocardiogram for cardiac ejection fraction (EC) and electrocardiogram were obtained before the first and every three cycles of treatment. Patients were taken off the study if LVEF decreased more than 20% from baseline value or in case of symptomatic congestive heart failure.

Health-related quality of life (HRQOL) was assessed using the EORTC QLQ-C30 (version 1), a 30-items questionnaire which incorporates the measurement of functional scales, global QoL scale, symptoms of cancer. The QLQ-C30 was self-administered at baseline, after three cycles and at the end of treatment. The QLQ C-30 responses were scored and analyzed according to algorithms in a scoring manual supplied by the EORTC. All raw scores were transformed from 0 to 100 scale; for the functional scales and the global QoL scale, higher scores indicated better functioning, whereas in the symptoms scale and items, higher score indicated a worsening of the symptoms.

Study analysis. Toxicity and response were determined according to WHO criteria [14]. Patients who received at least two cycles of combination treatment were evaluable for efficacy, and patients who received at least one dose of chemotherapy were evaluable for toxicity. The antitumor activity of the combination was assessed every three courses by radiological evaluation (magnetic resonance imaging, computed tomography scan, chest X-rays) or physical examination. Dose intensity was calculated according to the method of Hryniuk and Goodyear [15].

The primary end point of this phase II trial was to evaluate the antitumor activity (overall response rate, time to progression, overall survival) and toxicity profile of PLD in combination with gemcitabine in chemo-naïve or pretreated patients with MBC. Complete response was defined as the disappearance of all measurable and non-measurable disease; partial response as the decrease of 50% of the sum of the products of the bidimensionally perpendicular diameters of all measurable disease, no progression of assessable disease, no new lesions; progressive disease, the increasing of 25% of the sum of the products of the bidimensionally measurable lesion, appearance of any new lesion/site, worsening of any assessable disease or failure to return for evaluation because of death or worsening condition (unless clearly unrelated to this cancer); stable disease, neither complete response, partial response or progressive disease criteria met. The duration of overall response was measured for responses from treatment start to the first date that recurrent or progressive disease was objectively documented. Time to progression was defined as the period of time from the first day of treatment to the time when disease progression or relapse was clearly documented. Survival was defined as the interval from the first day of treatment to the date of patients death. The safety and tolerability of the combination were evaluated by changes in hematological parameters and other clinical laboratory values, physical examination, and the frequency and severity of adverse events.

Statistical analysis. Patients were consecutively accrued in a phase II single-center study. The accrual goal called for a total of 49 subjects with patients required to have at least one prior chemotherapy regimen for MBC. A total of 18 responders among 49 patients were considered adequate to justify further study of the regimen. With this single-stage study design, the sample size of 49 patients is sufficient to give a 90% probability of rejecting a baseline response rate of 25% with an exact 5% one-sided significance test when the true response rate is 45%. Calculation of confidence intervals was performed according to standard methods. Time to event was estimated with the Kaplan–Meier method.

Results

Fifty women were enrolled into the trial between February 2003 and April 2004. Patients' characteristics are shown in Table 1. The median age was 55 years (range 33–73), and the median Performance Status (WHO) at study entry was 0 (range 0–2). Most of the patients (37 patients, 74%) had visceral site as dominant metastatic localization. Thirty-seven patients (74%) had previously received adjuvant chemotherapy, while 15 (30%) and 8 (16%) patients had previously received 1 or ≥2 chemotherapy options for metastatic disease, respectively. Twenty-four patients and 6 patients (30 patients total) had previously received anthracycline-based regimen in the neoadjuvant/adjuvant and metastatic settings, respectively. Median dose of previously administered doxorubicin or epirubicin was 300 mg/m^2 (range 240–360) and 450 mg/m^2 (range 240–480), respectively. The median number of courses administered was 6 (range 1–12).

Efficacy. The activity of the combination is shown in Table 2. Four patients were not evaluable for efficacy [three patients had early treatment suspension due to toxicity (one hypertransaminasemia, one adverse reaction with cutaneous erythema during the first administration of PLD, one early death) and one patient early death for progressive disease]. At intent-to-treat analysis, 2 patients obtained a complete response (4%) and 20 patients a partial response (40%) (overall response rate 44%, CI 95%: 30.2–57.8), while stable disease was seen in 12 patients (24%) and progressive disease in 12 patients (24%). The median duration of response was 7 months (range 4–13). Clinical benefit (ORR and stable disease ≥6 months) was 60% and the median duration of clinical benefit was 8 months (range 4–13). The time to

Table 1 Characteristics of the patients

Patients	*N* (%)
Age, median years (range)	55 (33–73)
WHO performance status	
0	33 (66)
1	16 (32)
2	1 (2)
Premenopausal status	9 (18)
Postmenopausal status	41 (82)
Receptor status	
ER + PgR +	19 (38)
ER + PgR −	6 (12)
ER − PgR +	2 (4)
ER − PgR −	20 (40)
Unknown	3 (6)
HER2 expression by IHC[a]	
HER2 negative or 1 +	23 (46)
HER2 2 +	11[a] (22)
HER2 3 +	10 (20)
Unknown	6 (12)
Dominant metastatic sites	
Liver	24 (48)
Lung	13 (26)
Bone	3 (6)
Soft tissues	10 (20)
Median number of metastatic sites (range)	2 (1–4)
Previous chemotherapy	
Neoadjuvant/adjuvant setting	37 (74)
Anthracycline-including regimen	24 (69)
Metastatic setting	23 (46)
Anthracycline-including regimen	6 (26)
Previous median anthracycline dose (mg/m^2)	
Doxorubicin (range mg/m^2)	300 (240–360)
Epirubicin (range mg/m ^{2})	450 (240–480)

HER2 Human epidermal growth factor receptor, *IHC* Immunohistochemistry
[a]Nine of them with amplification at DNA level detected by FISH

progression was 7 months (CI 95%: 6–8) and at a median follow-up of 10 months (1–17), 79.4% patients were alive at 1 year.

The two complete responses had a duration of 13 and 10 months, respectively. Among the 22 responders, 14 (63.6%) were chemo-naïve for metastatic disease, 5 (22.7%) had been previously treated with first-line chemotherapy and 3 (13.7%) had previously received ≥2 chemotherapy lines for metastatic disease (Table 3). Thirty of 50 patients were previously treated with anthracycline-based chemotherapy. Among them, 1 (3.3%) and 13 (43.3%) achieved a complete and partial response, respectively, 7 (23.3%) stable disease, while 7 (23.3%) showed progressive disease; two patients were not evaluable for activity (Table 4). There was no significant difference between anthracycline-treated and anthracycline-naïve patients ($P = 0.12$).

Twenty-one women had HER2-overexpressing (HER2 2 + /3 + Dako Herceptest) breast tumors, while in other 23 patients breast cancer did not overexpress HER2 (0/1 +). HER2-overexpression score of 2 + showed amplification at the DNA level detected by FISH in 9 out of 11 tumors (Table 5). In six patients HER2 expression was not assessed. Concerning patients with HER2 2 + /FISH amplification and HER2 3 + tumors (19 patients), 68.4% experienced a response [13 patients (1 CR and 12 PRs)], 15.8% (3 patients) had evidence of stable disease, 10.5% (2 patients) had progression of disease and 1 patient was not assessable for activity. Conversely, among women with HER2-negative tumors (23 patients), the ORR was lower than observed in HER2-positive cases (26%), with a rate of stability and progression disease of 39.1% (9 patients) and 26% (6 patients), respectively. These results resembled those previously published by our group [16].

Toxicity. All the patients were assessable for toxicity. The grades of treatment-related toxicity encountered in the study are listed in Table 6. A total of 279 cycles were administered. The most common toxicity was neutropenia; grades 3 and 4 neutropenia were observed in 13 (26%) and 2 (4%) patients, respectively. Grade 3–4 febrile neutropenia was reported in two patients (4%). These events were not complicated by sepsis and resolved with G-CSF support, which was used in 5% of patients. The median time to neutropenia occurrence was 14 days (range 8–16). Grade 3 anemia and thrombocytopenia were observed in three (6%) and two (4%) patients, respectively, and in both cases transfusions were not needed. Only one patient needed hospitalization for grade 3 anemia and thrombocytopenia and for

Table 2 Activity of the combination

Patients	Intent to treat ($n = 50$)		Assessable ($n = 46$)	
	N	%	*N*	%
Complete response	2	4	2	4.3
Partial response	20	40	20	43.5
Overall response	22	44 (CI 95%: 30.2–57.8)	22	47.8 (CI 95%: 33.4–62.3)
Stable disease	12	24	12	26.1
Progression	12	24	12	26.1
Non-evaluable	4	8	4	–
Duration response	7 months (range 4–13)			
Median time to progression	7 months (CI 95%: 6–8)			
1 year survival	79.4%			

grade 4 neutropenia without fever and after the toxicity recovery she died of pulmonary embolism as complication of disease, as demonstrated by the autopsy.

Non-hematological toxicity was mild. Gastrointestinal side effects such as nausea/vomiting or stomatitis were uncommon: grade 3 emesis was observed in one patient (2%), while grade 2–3 mucositis occurred in six (12%) and five (10%) patients, respectively. Increase of transaminases' levels was frequently observed: six (12%) and three (6%) patients had grades 2 and 3 hypertransaminasemia, respectively. In one case the treatment was stopped according to protocol, since the recovery from toxicity did not occur within 2 weeks. Grade 3 peripheral neuropathy affected only one patient (2%) who had preexisting symptoms from prior taxane-based treatments. Grade 2–3 palmar–plantar erythrodysesthesia was observed in three patients (6%), and only one needed support medication with pyridoxine.

One patient experimented an adverse reaction with cutaneous erythema (face and hands), arterial hypertension and tachycardia 20 min after the first administration of PLD; the infusion was stopped and the patient was treated with steroids and H2-antihistamines with the regression of symptoms described. The patient refused to continue the treatment. Complete alopecia was observed only in one patient (2%) and partial alopecia in three patients (6%). Mild, moderate and severe asthenia was observed in 11 (22%), 6 (12%) and 3 (6%) patients, respectively.

Two patients (4%) had cardiac toxicity as observed after the end of the sixth and eighth courses, respectively. One patient, not previously treated with anthracyclines, had an LVEF reduction of 17% from the basal value; the other patient, who previously received a dose of epirubicin of 450 mg/m^2, had an LVEF reduction of 26% from the basal value. In both cases LVEF value was superior to 50%. No patient had symptomatic congestive heart failure. Cardiac toxicity in both patients recovered after 6 months from the end of the study.

The main causes for dose modification were neutropenia and transaminases elevation: in 11 (4%) cycles the dose of PLD and gemcitabine on day 1 was reduced by 25% for grade 3–4 neutropenia. The day 8 dose of gemcitabine was omitted in 20 (7%) cycles for severe neutropenia and the dose was reduced by 25 and 50% in 18 (6.4%) and 15 cycles (5%), respectively, due to neutropenia and/or transaminases, increase. The delivered dose of PLD and gemcitabine was 92 and 86% of the projected dose respectively.

Table 3 Response according to previous lines for metastatic disease

Previous chemotherapy for metastatic disease	No of. patients	Percentage
Naïve	14	63.6
First-line	5	22.7
≥ 2 lines	3	13.7
Total	22	100

Quality of life. Of the 50 patients enrolled in the study, 41 (82%) completed the QLQ-C30 questionnaire at baseline and the number of patients available for HRQOL analysis (those who had baseline and at least one evaluation at the end of the study) was 36 (72%). During treatment the functional scales (physical, role and social functioning) were not significantly improved comparing baseline time and the end of treatment ($P = 0.58$), while global QoL scale and symptoms scale resulted in significant amelioration comparing baseline time with the end of treatment ($P = 0.003$).

Discussion

Based on the previous results of phase I/II trials on PLD in combination with gemcitabine for MBC [12, 13], our phase II study was conducted to further evaluate the activity and tolerability of this regimen even in patients pretreated for metastatic disease (46% in our series).

In a phase I study, Rivera et al. studied PLD in combination with gemcitabine to determine the maximum-tolerated dose and toxicity profile of this combination [12]. The regimen selected for use in the phase II study was PLD, 24 mg/m^2 on day 1, plus gemcitabine, 800 mg/m^2 on days 1 and 8, every 21 days. Results indicated that this regimen was both effective (overall response rate of 33.3%) and well tolerated. The subsequent phase II study was conducted to evaluate the efficacy and safety of PLD in combination with gemcitabine as first-line treatment for MBC (12). Of the 49 patients enrolled, 28 (57%) had undergone prior adjuvant chemotherapy; 19 of those patients had received prior anthracycline regimens. An overall response rate of 52% was observed: 3 complete responses and 21 partial responses; 2 complete and 9 partial responses were observed in patients with previous anthracycline exposure. The median duration of response was 5.6 months, the median time to progression was 4.5 months and the median overall survival was 16.1 months.

Considering our series, the overall response rate (intent to treat) was 44% (2 patients obtained a CR and 20 patients a PR). The median duration of response was 7 months, the median TTP was 7 months, and at 1 year 79.4% patients were alive. It must be highlighted that in our study, in contrast to Rivera's series, 23 out of 50 patients had been previously treated with chemotherapy and that 30 out of 50 patients had received anthracycline-based chemotherapy. Among them, 13 (43.3%) reached a partial response.

The most common toxicity observed in our study was hematological, with neutropenia and thrombocytopenia being the most frequent. Concerning grade 3 or 4 hematological toxicity, no substantial differences were observed comparing our study with that by Rivera et al. [13], with the exception of thrombocytopenia (grade 3, 4% in our series vs 25.5% in Rivera's study). Neutropenic fever and/or events were not commonly observed with this regimen in both studies. Hospitaliza-

Table 4 Response in subgroup of anthracycline-naïve and anthracycline-pretreated patients

Patients	Anthracycline-treated		Anthracycline-naïve	
	No. of patients	Percentage	No. of patients	Percentage
Complete response	1	3.3	2	5
Partial response	13	43.3	7	35
Overall response	14	46.6	9	40
Stable disease	7	23.3	5	25
Progression	7	23.3	5	25
Nonevaluable	2	6.7	2	10
Total	30	100	20	100

Table 5 Response and HER2 expression

	HER2 2+ (FISH+)/3+ Patients: 19		HER2 -/1+ Patients: 23	
	Number	Percentage	Number	Percentage
Complete response	1	5.3	1	4.3
Partial response	12	63.1	5	21.7
Overall response	13	68.4	6	26.0
Stable disease	3	15.8	9	39.1
Progression	2	10.5	6	26.1
Not evaluable	1	5.3	2	8.7
Total	19	100	23	100

[a]Six patients with HER2 unknown

Table 6 Toxicity (WHO) per patients (total patients 50)

Toxicity	G1 (%)	G2 (%)	G3 (%)	G4 (%)
Leucopenia	1 (2)	6 (12)	4 (8)	–
Neutropenia	1 (2)	9 (18)	13 (26)	2 (4)
Anemia	4 (8)	6 (12)	3 (6)	–
Thrombocytopenia	1 (2)	2 (4)	2 (4)	–
Neutropenic fever	–	–	1 (2)	1 (2)
Mucositis	3 (6)	6 (12)	5 (10)	–
Liver[a]	5 (8)	6 (12)	3 (6)	–
PEE	1 (2)	2 (4)	1 (2)	–
Mialgias	1 (2)	–	–	–
Peripheral neuropathy	1 (2)	–	1 (2)	–
Diarrhea	–	2 (4)	1 (2)	–
Nausea/vomiting	4 (8)	2 (4)	1 (2)	–
Hypersensitivity	–	–	–	1(2)

[a]Increase of transaminase levels
PEE Palmar–plantar erythro dysesthesia

tions during our study were very infrequent (one) and only 5% of the patients required the use of G-CSF. There were no treatment-related deaths in this study. Non-hematological toxicity was not predominant during the study. Discontinuation of treatment was not usually related to this type of toxicity. According to Rivera's study [13], the most common grade 3 non-hematological toxicities were nausea and vomiting (10.6%), fatigue (25.5%), stomatitis (8.5%) and hand–foot syndrome (6.3%). Alopecia (requiring a wig) occurred in only 2/46 patients. In our study, non-hematological toxicity resembled that reported by Rivera et al. [13], with the exception of a more severe stomatitis (grade 2: 12% and grade 3: 10%) and an increase of transaminase levels (grade 2: 12% and grade 3: 6%). Hand–foot syndrome/palmar–plantar erythrodysesthesia (HFS/PPE) was mild, infrequent, and did not cause patients to stop their treatment. Grade 2–3 HFS/PPE was observed in three patients (6%), and only one case needed support with pyridoxine. This can be explained in part by the fact that we used lower doses of the drug than those we would normally expect, to produce this particular toxicity (50 mg/m^2). The modest toxicity profile of PLD in combination with gemcitabine should be regarded as an undeniable advantage of this regimen, since 46% of our patients, in variance with Rivera's study, had been previously treated with chemotherapy.

Doxorubicin displays an excellent antitumor activity in breast cancer but its use in clinical practice is limited by drug-associated toxicities, particularly cardiotoxicity. These potential cardiac effects often limit repeated use of the drug, especially in the elderly and

in those patients with a history of cardiac disease; therefore the recommended lifetime cumulative dose of doxorubicin is 450–550 mg/m^2. Stealth liposomal doxorubicin has been reported to have significantly less cardiac toxicity compared to standard doxorubicin [5, 17, 18]. The goal of liposomal encapsulation of doxorubicin is to alter the tissue distribution and pharmacokinetics of the drug to increase its therapeutic index. Previous studies have demonstrated improved cardiac safety with PLD vs conventional doxorubicin, even at doses exceeding the recommended maximum lifetime cumulative doxorubicin dose [19–21]. In the study by Rivera et al. [13], one patient experienced a transient 21% decrease in LVEF from baseline after a cumulative PLD dose of approximately 236 mg/m^2. That patient had predisposing risk factors (>65 years of age, prior radiation to the left chest wall, history of idiopathic pulmonary fibrosis). She was taken off the study, and her cardiac function recovered within 2 months. Another patient had a decrease in LVEF from 69.8 to 55% but remained asymptomatic with no further decline in ejection fraction while on study. None of these two patients had clinical evidence of congestive heart failure. In our series, two patients had a cardiac toxicity: one patient, who previously received of 450 mg/m^2 of epirubicin, had a FEV reduction of 26% from the basal value, while the other one, not previously treated with anthracyclines, showed an LFEV reduction of 16.9% from the basal value. In both cases patients were ≥65 years of age and LFEV values remained above the threshold of 50%. None of them had symptomatic congestive heart failure. Cardiac toxicity in both patients recovered after about 6 months from the end of the study. Thus, considering the low incidence of cardiotoxicity and the comparable response rate between anthracycline-naïve and anthracycline-pretreated patients, re-treating these patients with another PLD-containing combination seems reasonable. Reduced cardiotoxicity, with significantly fewer clinical cardiac events, has been reported in a study comparing PLD with conventional doxorubicin in patients with MBC [19]. However, in our study the occurrence of cardiotoxicity was infrequent probably because lower doses of PLD were administered. Thus, in chemo-naïve patients for metastatic disease we did not reach a high enough cumulative dose level of total (free and liposomal) doxorubicin so as to expect any serious and irreversible cardiac toxicity. However, these findings become very interesting if we consider that 30 women had been previously given anthracyclines at a median dose of 300 mg/m^2 of doxorubicin (higher than that reported by Rivera et al.) and 450 mg/m^2 of epirubicin.

There was a statistically significant improvement of fatigue comparing the baseline time with the end of study treatment and a good amelioration in global QoL. This improvement was seen from the third course of treatment in half of the patients assessable for HRQOL analysis. The reasons for improvement in the global QoL and fatigue in our series of patients are the good response of the metastatic sites (in fact the majority of patients who had a better QoL were responders), the low incidence of total alopecia and the good tolerability of the regimen without frequent and important reduction of the hemoglobin value.

The predictive value of HER2 expression regarding response to therapeutic interventions in breast cancer is still controversial, even though chemotherapy trials have supported an interaction between HER2 overexpression and chemotherapy sensitivity (CMF resistance and doxorubicin sensitivity) [22–26]. By contrast, Pegram et al. [27] tested this hypothesis by transfecting four breast cancer cell lines with HER2 and then exposing them to doxorubicin in vitro. No alteration in chemosensitivity was observed in any of the transfected breast cancer cell lines compared with the parent cell lines or in a related in vivo nude mouse xenograft model. These observations argue against a direct role for HER2 amplification in anthracycline sensitivity [28]. In our study, concerning patients with HER2-positive tumors, 68% experienced a response, 16% had evidence of stable disease, while 10.5% had disease progression. Conversely, among women with HER2-negative tumors, the ORR was lower than that observed in HER2-positive cases with an ORR of 26% and a rate of stability and progressive disease of 39 and 26%, respectively. Our findings seem to support, even considering the low number of patients recruited, a positive interaction between HER2 overexpression and sensitivity to PLD [16], substantially confirming the sensitivity of HER2-positive tumors to doxorubicin previously described by some authors [29–32]. Further large-scale investigations are needed to confirm our observations. However, considering the large use of liposomal doxorubicin and gemcitabine both as single agents or in combination in the treatment of advanced breast cancer, particularly in elderly patients, it would be useful to predict the response to chemotherapy according to the HER2 expression. PLD has demonstrated efficacy as a single agent in patients with metastatic or recurrent breast cancer, with objective response rates ranging from 31 to 33% [33, 34]. In addition, it has been reported that trastuzumab may be combined with EC with manageable cardiotoxicity and promising efficacy [35]. Recent data indicate that adding trastuzumab to chemotherapy (four cycles of paclitaxel followed by four cycles of fluorouracil, epirubicin and cyclophosphamide) significantly increases the pathologic complete response without clinical congestive heart failure [36]. These findings provide the rationale for evaluating the combination of trastuzumab with liposomal doxorubicin, considering its similar efficacy profile and improved safety profile in comparison with conventional doxorubicin. Thus, since trastuzumab plus PLD have been reported to be active and safe [37], our combination would become an attractive option when anthracyclines are contraindicated, such as in HER2-positive cases that receive trastuzumab. Among

the nanoscale drug delivery systems, liposome-based agents, particularly liposomal anthracyclines, provide an ideal foundation for future liposome therapeutics [38]. Thus, newer approaches that conjugate MAb fragments or other ligands to liposomes can achieve true molecular targeting and appear highly promising.

Breast cancer patients previously exposed to multiple chemotherapy regimens tend to become quite sensitive to the side effects related to additional treatment and response to treatment tends to be low. Few studies in the literature report results with the use of PLD in combination with gemcitabine in patients with MBC. Our efficacy results compare favorably in terms of response rates with those reported in other phase I/II studies, supporting the clinical utility of PLD plus gemcitabine combination therapy in patients with MBC [12, 13, 39, 40]. In addition, this combination is well tolerated and shows a favorable toxicity profile. In fact, an high number of patients derived clinical benefit from treatment. Finally, because of the modest and non-overlapping toxicity profiles of both PLD and gemcitabine, this combination can be regarded as a reliable therapeutic option for patients who have failed previous treatments, including anthracycline, for MBC.

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