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CLINICAL TRIAL

Pegylated liposomal doxorubicin and trastuzumab as 1st and 2nd line therapy in her2/neu positive metastatic breast cancer: a multicenter phase II trial

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Abstract The combination therapy of doxorubicin and trastuzumab has been proven to be highly effective for metastatic breast cancer (MBC) patients with Her2/neu over-expressing tumors. However, this regimen is characterized by frequent cardiac toxicity, occurring in 27% of all treated patients and aggravating when the two substances are given concurrently. Pegylated liposomal doxorubicin (PLD) as a single agent reduces significantly cardiac toxicity and maintains efficacy compared to conventional doxorubicin. This prospective open labeled, multicenter phase II study assessed the potential cardiotoxicity and efficacy of PLD and trastuzumab as first and second line combination therapy in Her2/neu over-expressing MBC patients. Patients with Her2 over-expressing, measurable MBC with a baseline left ventricular ejection fraction (LVEF) $\geq$50% were treated with PLD 40 mg/m^2 every 4 weeks for 6 up to 9 cycles and weekly trastuzumab (4 mg/kg loading dose, then 2 mg/kg). Cardiotoxicity was defined as the appearance of clinical signs or symptoms of congestive heart failure in combination with a decrease in LVEF $\leq$44% or $\geq$10 units below the normal value of 50% in the obligatory, subsequently performed transthoracic echocardiography. Due to conflicting interests, the planned accrual goal of 30 patients was not reached. Finally 16 patients were enrolled. Ten patients presented with more than one metastatic site and six of them were in second-line therapy. The median LVEF in the study cohort was 66.1 $\pm$ 8.68% at baseline, 62.7 $\pm$ 5.11% after 6 cycles of therapy, 64.4 $\pm$ 7.61% at the first follow up and did not change significantly (61.0 $\pm$ 5.56% even at the 5th follow-up). Six out of 12 assessable patients (50.0%) demonstrated a clinical benefit and after a median follow-up of 15.4 months a median progression free survival of 9.67 and a median overall survival of 16.23 months. Non-cardiac side effects were mild with only 3 CTC grade 3 events of 247 treatment cycles (1.2%) and no grade 4 toxicities. The combination of PLD and trastuzumab in patients with Her2/neu over-expressing metastatic breast cancer is a safe, feasible and effective therapy. However, cardiac function should be monitored at close intervals. Due to the promising clinical response rates and mild toxicity profile in this prognostically unfavorable group, this combination therapy should be evaluated in larger studies.

Keywords Pegylated doxorubicin · Trastuzumab · Metastatic breast cancer · Cardiotoxicity

Introduction

The Her2/neu gene is amplified and the p185HER2 protein overexpressed in 20 to 30% of human breast cancers [1]. This over-expression is correlated with an induced mitosis and proliferation rate of tumor cells and Her2 positive breast cancers are more likely to present at an advanced stage and are associated with poorer disease-free and overall survival in the absence of trastuzumab therapy compared with Her2 negative breast cancers [2]. Trastuzumab increases the clinical benefit of first-line

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chemotherapy in metastatic breast cancer (MBC) which overexpress Her2/neu [3] and moreover, in combination with chemotherapy, in the first-line setting improves response-rates, time-to-progression, time-to-treatment-failure and median overall survival [4]. The monoclonal antibody trastuzumab is specifically directed against the Her2/neu receptor and associated with an increased risk of cardiac toxicity ranging from asymptomatic decreases of the left ventricular ejection fraction (LVEF) up to chronic heart failure. Generally, trastuzumab mediated cardiac toxicity tends to be most frequently of mild nature, clinically manageable, reversible and under close clinical supervision not an absolute contraindication for continuation of trastuzumab therapy and has to be seen in contrast to the anthracycline mediated irreversible cardiotoxicity [5].

Pegylated liposomal doxorubicin (PLD) was developed to improve the toxicity profile by encapsulating doxorubicin hydrochloride in pegylated liposomes while simultaneously maintaining the antineoplastic effects. In a phase III trial, PLD has demonstrated as a first line therapy a similar efficacy to conventional doxorubicin. Moreover, due to its pharmacologic formulation, PLD is characterized by a more moderate toxicity profile in comparison to conventional doxorubicin. When administrating PLD, specific attention needs to be drawn to a higher incidence of stomatits and palmar-plantar erythrodysesthesia (painful redness, swelling, blistering or ulceration of the palms and soles). Concerning cardiac toxicity, a lower rate of LVEF decreases (10 vs. 48%, respectively) and the lack of reaching a critical cumulative dose of PLD for cardiac toxicity was observed [6].

The rationale for the combination of cytotoxic agents with trastuzumab is based on the highly synergistic effects of these two treatment strategies observed in many in vitro studies suggesting also a high potential for an existing clinical benefit [7–9]. Slamon et al. described in their landmark paper the highly significant reduction in the risk of beast cancer death by 20% due to the combination therapy of trastuzumab plus anthracycline/cyclophosphamide chemotherapy. However, this high potential clinical benefit must be weighed against the potential cardiotoxic profile of both agents, especially when given concurrently [10]. In their study, Slamon et al. described a 27% incidence of cardiac dysfunction for the combination therapy of trastuzumab and doxorubicin compared to 13% for the combination of trastuzumab and paclitaxel. The only significant risk factor for cardiac dysfunction in this study was the age of the patients [11]. Reviewing the actual literature, independent risk factors for anthraycline and trastuzumab associated cardiac toxicity include age (>65 years), obesity (>27 kg/m^2), hypertension, hypercholesterolemia, pre-existing cardiac diseases (incl. borderline LVEF) and diabetes mellitus [12].

Two clinical studies have evaluated the cardiac safety of PLD plus trastuzumab in MBC. In 2006, a multicenter phase II study of Chia et al. was conducted to assess the rate of cardiotoxicity and clinical benefit of first line PLD combined with trastuzumab in 30 patients with HER2-positive MBC. For the investigated patient cohort the authors reported a median progession free survial (PFS) of 12 months with mild cardiotoxicity limited to asymptomatic declines in LVEF [13].

In 2007, Andreopoulou et al. [14] conducted a second prospective phase II trial recruiting twelve patients. Three patients experienced grade 2 left ventricular dysfunction.

A different preparation of liposomal-encapsulated doxorubicin (non-pegylated, Myocet™; D-99) has also shown a clinical benefit combined with a low cardiotoxicity rate when combined with trastuzumab [15, 16].

Our prospective, open labeled, phase II multicenter trial was conducted to evaluate the potential cardiotoxicity and efficacy of PLD and trastuzumab as a first and second line combination therapy in Her2/neu over-expressing MBC.

Patients and methods

Study design

Our trial was an open label, prospective multicenter phase II study evaluating the safety and efficacy of the combination PLD and trastuzumab in the first and second line therapy of MBC.

Based on pre-clinical data the hypothesis of this study was a clinical benefit of this regimen combined with an acceptable cardiac safety. The primary objective was to assess the potential cardiotoxicity. Secondary endpoints were response rates (RR) of patients with complete remission (CR), partial remission (PR), stable disease (SD), progressive disease (PD), PFS and overall survival (OS).

Patients

Within a period of 24 months 15 patients with HER2 positive histologically confirmed invasive breast cancer and metastatic disease were enrolled. Her2/neu positivity required either immunohistochemic staining of 3 + or positivity by fluorescence in situ hybridization (FISH) or an immunohistochemical score 2 + with a confirmatory FISH test.

In patients with measurable and/or evaluable lesions and adequate organ function, including no prior history of cardiac disease, PLD was administered for 6 or 9 cycles, trastuzumab was administered until progression.

Prior chemotherapy or endocrine therapy for 1st line treatment of metastatic disease was permitted, but no prior

high dose chemotherapy, radiation therapy of the mediastinum nor therapy with trastuzumab. Prior exposure to anthracyclines in the adjuvant or metastatic setting to a maximum cumulative dose of $\leq$400 mg/m^2 for doxorubicin and $\leq$600 mg/m^2 for epirubicin, respectively, was permitted with no obligatory minimum treatment free interval. Patients with a prior history of cardiac disease were not allowed to enter the study, same as patients with clinical sings of heart failure, LVEF of $\leq$50%, myocarditis/pericarditis, arrhythmia requiring treatment, coronary heart disease, hypertonus ($\geq$140/$\geq$95 mmHg), clinically apparent heart valve disease, prior mediastinal irradiation or prior trastuzumab therapy. Inclusion criteria were: Karnofsky-Index $\geq$60%, life expectancy longer than 12 weeks, baseline neutrophils $\geq 1.5 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, hemoglobine $\geq$9 g/dl, GOT/GPT $\geq$3 times upper limit of normal, creatinine 1.5 times upper limit of normal, alkaline phosphatase $\geq$3 times upper limit of normal (on case of bone metastasis: $\geq$5 times upper limit of normal) and a baseline LVEF of $\geq$50%. The follow-up time interval was every 3 months.

The study was in accordance with the local research ethic boards at each participating center (Central approval No. 313/2000). A written informed consent of each patient was obtained.

Treatment

Patients were treated according to the protocol and received 40 mg/m^2 PLD i.v. bolus on day 1 every 4 weeks for up to 9 cycles. Trastuzumab with a loading dose of 4 mg/kg was administered on day 2, followed by weekly administration of 2 mg/kg in combination with PLD, until tumor progression.

Cardiac toxicity

To assess cardiotoxicity, patients were asked for signs or clinical symptoms of CHF at baseline and during follow up. In addition, an electrocardiogram (ECG) and a transthoracic echocardiographic examination were performed at baseline, and 3–8 days after each cycle of PLD and trastuzumab therapy, and every 3 months during trastuzumab therapy alone. Echocardiography allowed the assessment of the diameter of the left ventricle and left atrium and the estimation of the global and regional left ventricular systolic function and of LVEF. Regional wall motion analysis was semiquantitative. Left ventricular volume and ejection fraction was determined by the Simpson's method using a combination of an apical four-chamber view and an apical two-chamber view. Valvular heart diseases were evaluated by the Doppler method and color flow imaging. Mitral inflow velocity was used for the assessment of diastolic filling. An E/A-velocity ratio $\leq$1 implicated a diastolic dysfunction.

Cardiotoxicity was defined as the appearance of clinical signs and symptoms of CHF (sudden limitation of activity, dyspnoea, tachycardia, inspiratory crackles e.g.) in combination with a decrease in LVEF $\leq$44% or $\geq$10 units below the normal value of 50%. A transthoracic echocardiography was obligatory in any case of clinical signs or symptoms of CHF. Furthermore, this was also considered in case of a decrease of $\geq$15 units, irrespective of the total LVEF value.

Non-cardiac toxicities

Adverse events were graded according to NCI-CTC version 2.0. Investigators reported adverse events severity as usual whether the adverse event was probably, possibly, or not related PLD or trastuzumab.

Efficacy

Efficacy was monitored by 4 weekly reevaluation of bidimensionally measurable lesions. Response determination was in accordance to RECIST criteria. In case of PD the patient was excluded from the study.

Statistical analysis

Response rates, adverse events, LVEF values and changes in laboratory parameters were summarized and tabulated. Progression free survival and OS were calculated with Kaplan–Meier estimates.

Results

The trial was unfortunately terminated after the inclusion of 16 of the planned 30 patients due to conflicting interests in the sponsorship. A total of 16 patients (median age, 63 years; range 27–68 years) were enrolled in six centers. Seven patients received chemotherapy and seven patients endocrine treatment in the adjuvant setting. The majority had MBC to visceral organs with 68% of the patients presenting more than one metastatic site. Five patients (33%) received prior systemic treatment for MBC with two patients having had prior anthracycline-containing chemotherapy and three patients prior endocrine treatment (Table 1).

Changes in cardiac function

In the follow-up period, three patients developed minor ECG changes without clinical significance. A first degree

Table 1 Baseline patient characteristics

Characteristic	Numbers	Percentage (%)
Patients enrolled	16	100
Response of assessable patients	15	93.8
Median age	63 (37–68)	
Site of metastases		
Liver	8	50
Lung	8	50
Bone	8	50
Lymph nodes	7	43.75
Mediastinum	2	12.5
Pleural	1	6.25
Skin	1	6.25
Local reccurence	1	6.25
Number of sites		
1	5	31.25
2	4	25.0
3	4	25.0
4	1	6.25
5	2	12.5
Prior surgery		
Curative	14	87.5
Palliative	4	25
Prior endocrine treatment		
Adjuvant	7	43.75
1st line	3	18.75
Radiation		
Adjuvant	7	43.75
1st line	3	18.75

AV-blockage was observed in one patient (5th follow up) and two patients developed slight ST-deviation without clinical symptoms (1st and 5th follow up, respectively). With regard to the left ventricular function, the mean LVEF was 66.1 ± 8.68% at baseline, 62.7 ± 5.11% after 6 month cycles of therapy and 64.4 ± 7.61% at the first follow up. This value did not change significantly until the last examination (mean LVEF 61.0 ± 5.56%; Fig. 1).

Three patients developed moderate to severe echocardiographic changes of the left ventricular function: the first patient developed a decrease in LVEF from 69 (baseline) to 55% (5th follow up) without any clinical signs or symptoms of CHF. The decline was predominantly during the follow-up period. The second patient decreased continuously from 70 at baseline to 60% at 5th follow up. In this patient, a hypokinetic left ventricle was moreover revealed. However, this patient was also asymptomatical. A severe decrease of the LVEF was diagnosed in the third patient. In this patient, a thrombus formation was visualized in the left ventricle. This patient started with a LVEF of 71% at baseline and was diagnosed with a LVEF of 35% at the 6th follow up examination. The patient died later because of hepatic failure. Changes of the E/A-velocity ratio as sign for a diastolic dysfunction were observed in three patients.

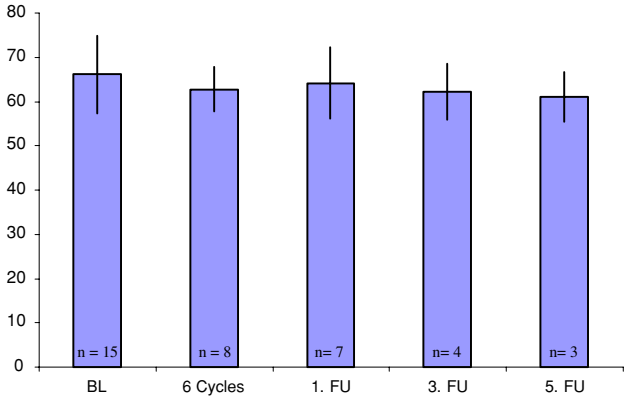

Fig. 1 Cardiac toxicity: mean left ventricular ejection fraction (LEVF) over time

Non-cardiac toxicity

Non-cardiac side effects were mild with the majority being grade 1 to grade 2 adverse events. The most frequent non-cardiac adverse events were alopecia ($n = 4$), hand-foot-syndrome ($n = 4$), and stomatitis ($n = 3$). Only 3 grade 3 events occurred of a total of 247 treatment cycles. These were hand-foot-syndrome ($n = 2$) and allergic reaction ($n = 1$). There were no grade 4 toxicities observed (Table 2).

Dose reduction and treatment discontinuation

Four patients received 6 cycles and four patients 9 cycles of PLD. Treatment discontinuation was due to PD in five and due to toxicity two patients. Dose reduction of PLD was

Table 2 Non-cardiac toxicity

Toxicity grade	CTC		
No of patients	I	II	III
Allergic reaction	0	1	1
Alopecia	2	2	0
Dysphagia	1	0	0
Dyspnoe	1	0	0
Hand foot syndrome	2	0	2
Nail changes	0	1	0
Nausea	1	1	0
Sensibility neuropathia	1	0	0
Stomatitis	2	1	0
Vomiting	1	0	0

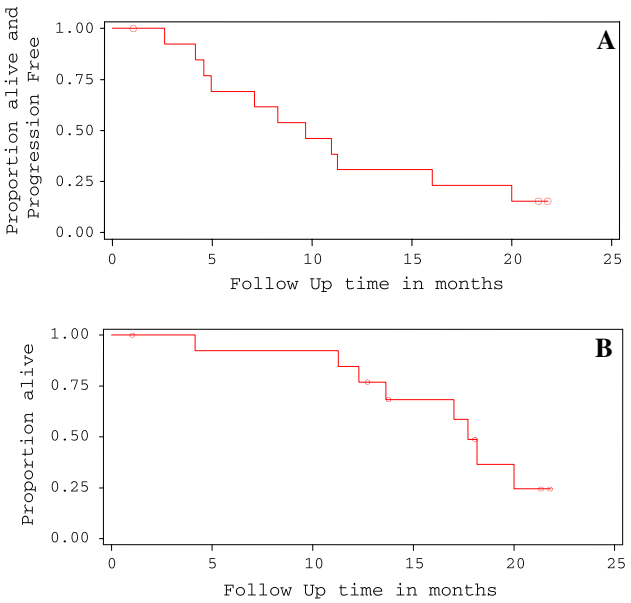

Fig. 2 **a** Progression-free survival; **b** overall survival

necessary in 1.2% (1/83 cycles) and an application delay was reported in 12.1% (10/83 cycles).

Efficacy

Twelve patients were assessable for efficacy while the final response determination was not assessed in four patients. Six patients (50.0%) demonstrated a clinical benefit with four patients (33.3%) for partial response (PR) and two patients (16.6%) for no change. Progessive disease was observed in six patients (50.0%). After a median follow-up of 15.4 months, the median PFS was 9.67 months and the median OS was 16.23 months, respectively (Fig. 2).

Discussion

Trastuzumab mediated cardiotoxicity was first described for patients with MBC and ranged from 13% in combination with paclitaxel up to 27% in combination with doxorubicin [11]. These observations raised many concerns despite the substantial benefits of trastuzumab to patients with Her2/neu positive breast cancer. In the adjuvant setting, with most of the treatment regimen containing anthracylines, the incidence rates for severe CHF in trastuzumab treated patients were much lower and ranged from 0.6 in the HERA to 4.1% in NSABP B-31 trial [17]. However, even in the adjuvant setting up to 14% of patients discontinued trastuzumab treatment due to asymptomatic but protocol sufficient decreases in LVEF. In a recent metanalysis pooled results from five randomized adjuvant trials of adjuvant trastuzumab revealed an increased likelihood of cardiac toxicity (HR 2.45, 1.89–3.16 95% CI, $P = 0.001$) in the trastuzumab treated patients [18]. In the neoadjuvant setting, preliminary results of the NOAH trial, presented at the 2007 ASCO Breast Cancer Symposium, revealed drops in LVEF of 15.7% for the trastuzumab regimen versus 11.5% for the non-trastuzumab arm in patients with locally advanced breast cancer receiving concomitantly 3 cycles of doxorubicin-paclitaxel (60 mg/m^2, 150 mg/m^2 q3w), 4 cycles of Paclitaxel (175 mg/m^2 q3w) and 3 cycles of cyclophosphamide/methotrexate/5-fluorouracil (C 600 mg/m^2, M 40 mg/m^2, F 600 mg/m^2) [19].

Anthracycline-induced cardiotoxicity is dose dependent with a critical cutoff of 550 mg/m^2 total doxorubicin dose. It manifests in several forms, ranging from acute arrhythmias and nonspecific electrocardiogram changes to decreases in LVEF. Beside age, hypertension, diabetes and coronary heart disease, trastuzumab treatment represents a significant risk factor for the development of anthracycline related cardiotoxicity [12, 18]. The most severe complication is cardiomyopathy leading potentially to a permanent cardiac damage [20]. For women >65 years of age receiving doxorubicin chemotherapy the reported hazard ratios for cardiomyopathy, CHF and heart death are 2.48, 1.38 and 1.35, respectively, and the relative risk remains elevated 5 years after therapy [21]. Irreversible damage to the myocytes by free radicals, so called Type I myocardial damage, is presumed to be the cause of anthracycline-induced cardiomyopathy [22, 23]. The development of encapsulated conventional anthracyclines, such as PLD have led to a reduction of the incidence and severity of cumulative dose-related cardiomyopathy while preserving antitumor activity [24–27]. Thus, PLD may offer patients the benefit of receiving higher cumulative doses (compared with conventional doxorubicin) over a longer period of time, ultimately resulting in greater drug exposure. In a head to head comparison PLD (50 mg/m^2, 4 weeks interval) was tested against doxorubicin (60 mg/m^2, 3 weeks interval) in a randomized phase III study as first-line therapy in MBC. The risk to develop cardiac toxicity was significantly higher within the doxorubicin arm than in the PLD arm (HR = 3.16; 95% CI 1.58–6.31; $P < 0.001$), while efficacy did not differ significantly (PFS: 6.9 months for PLD and 7.8 months for doxorubicin; OS: 21 and 22 months, respectively) [28].

Trastuzumab is also associated with an increased risk of cardiac toxicity ranging from asymptomatic decreases in the cardiac ejection fraction to CHF. This risk appears to be higher in patients receiving concurrent anthracyclines [29]. Slamon et al. [12] observed cardiotoxicity for trastuzumab in combination with anthracyclines in patients with MBC in approximately 27%. Approximately two-thirds of the cases were of New York Heart association Class III or IV

[30]. However, natural history of trastuzumab related cardiotoxicity differs significantly from the anthracycline induced one. It very rarely causes death, is mostly reversible without medication and not dose dependent. This led to the definition of the new category of Type II trastuzumab related myocardial dysfunction [22].

Our study evaluated the safety and efficacy of PLD in the combination with trastuzumab for Her2/neu overexpressing MBC, since this treatment modification may offer the known potential treatment benefit of an anthracyline-trastuzumab combination for Her2/neu overexpressing MBC and may help to avoid increased rates of cardiac toxicity.

The early termination clearly compromises the planned statistical power of our study. However, we feel that our findings add significantly to the existing observations of the safe cardiac profile of the tested combination therapy. The analysis of the safety data revealed a very low rate of non cardiac side effects with 1.2% of all treatment cycles and no grade 4 toxicity. The LVEF monitoring showed no significant overall changes during therapy as well as in the follow up situation (up to 15 months), underlining the cardiac safety of the treatment combination PLD and trastuzumab. However, three patients developed a decline in LVEF, two of them during the follow-up period. A clinically relevant and symptomatic decrease occurred in the third patient after the sixth scheduled follow-up LVEF with a striking decrease of LVEF to 35% combined with a thrombus in the left ventricle.

These data are in accordance to the two trials published by Chia et al. and Andreopoulou et al., [14, 15] which also assessed the cardiac toxicity of this regimen as first line therapy in MBC and reported only mild cardiac toxicity

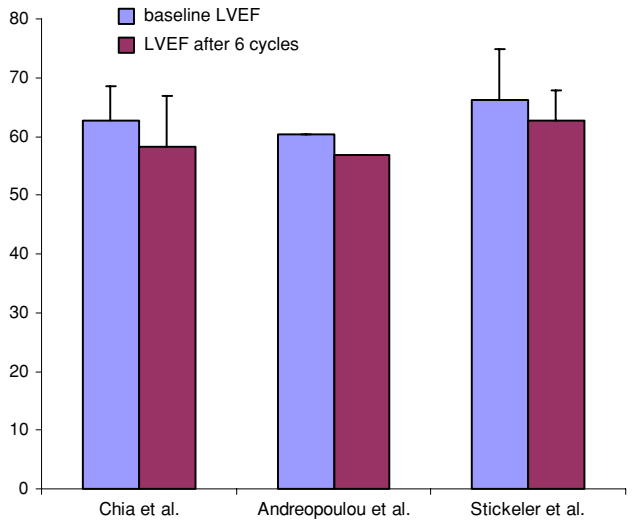

Fig. 3 Comparison of the LVEF decrease after 6 cycles of PLD and Trastuzumab in the three present phase II trials

(Fig. 3). Chia et al. [14] found in 10% of the treated patients an absolute decline in LVEF of $\geq$15% but no symptomatic CHF. These data underline the excellent cardiac tolerability of the treatment combination PLD and trastuzumab, especially since all of these patients received prior anthracycline containing adjuvant treatment. In the trial published by Andreopoulou, out of 12 pretreated patients, one discontinued treatment after grade 3 CHF, three patients experienced grade 2 left ventricular dysfunction, of whom two discontinued treatment. However, all of these three patients had preexisting cardiac risk factors [15]. Preliminary data of a multicenter controlled trial on the cardiac safety of doxetacel (60 mg/m^2) in combination with PLD (30 mg/m^2) alone (if Her2/neu negative) or with trastuzumab (2 mg/kg) if no anthracycline was given before in patients with MBC was presented at the 2003 ASCO Annual Meeting. The interim analysis confirms the findings of our study since it revealed an average drop of LVEF of 3.6% after 4 cycles in the arm of Doxetaxel/PLD with trastuzumab in 22 evaluable patients [31].

There is further evidence for a safe combination of anthracyclines and trastuzumab as a simultaneous treatment combination in the adjuvant as well as the metastatic setting. The GEPARQUATTRO neoadjuvant trial as well as the HERCULES trial for MBC investigated the cardiotoxicity for the combinatorial therapy of epirubicin plus trastuzumab. Both studies report no significant differences with regard to cardiac events with trastuzumab with no CHF and no cardiac related deaths [32, 33]. Taking this body of evidence into account we believe that our additional data support the statement on a safe cardiac toxicity profile of PLD plus trastuzumab. Furthermore our conclusions are supported by the large number of long term follow-up examinations in our study.

As a secondary endpoint we investigated the efficacy of the regimen. Six out of 12 assessable patients (50.0%) demonstrated a clinical benefit and after a median follow-up of 15.4 months a median PFS of 9.67 and a median OS of 16.23 months, respectively. Since our patient cohort was already pretreated with 40% in a second line situation for MBC and the majority of patients (68%) presented more than one metastatic site, these findings proof also with respect to the published literature the high activity of the regimen in a fist and second line setting. The data of our study are supported by Chia et al. showing response rates of up to 52% and median progression-free survival of 12.0 months and a median overall survival which was not reached at the time of publication with a follow-up of 13.9 months.

The therapeutic index is an important parameter for treatment choice in MBC. Within the above discussed limitations, our findings are in accordance with a growing body of evidence indicationg that the combination of PLD

and trastuzumab in patients with Her-2 over-expressing metastatic breast cancer is a safe and feasible therapy, without major side effects, particularly with respect to cardiac toxicity.

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