Lapatinib-plus-Pegylated Liposomal Doxorubicin in Advanced HER2-positive Breast Cancer Following Trastuzumab: A Phase II Trial

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Abstract. *Background: Trastuzumab, one important treatment option for HER2-positive metastatic breast cancer (MBC) is limited by its cardiotoxic potential. Lapatinib and pegylated liposomal doxorubicin (PLD) represent a cardiosparing alternative that can cross the blood brain barrier. This is important, because one third of breast cancer patients develop brain metastases. Patients and Methods: We included 24 patients with HER2-positive MBC progressing under trastuzumab. They received 1,250 mg lapatinib daily until progression plus PLD (40 mg/m^2) every 4 weeks for maximal 6 cycles. The primary end-point was the overall response rate (ORR). Secondary end-points were progression-free survival (PFS), overall survival (OS), 1-year PFS and 1-year OS rates. Results: ORR was 54%. Median PFS was 5.8 and median OS 23.3 months. The one-year PFS rate was 27% and 1-year OS rate 76%. Conclusion: Lapatinib-plus-PLD is active and safe in HER2- positive MBC, especially suitable for patients with cardiological risk or brain metastases.*

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Over-expression of human epidermal growth factor receptor type 2 (HER2) accounts for 15-25% of all breast cancer cases and is linked to a particularly aggressive course of disease (1). However, the introduction of HER2-directed therapy has significantly improved its prognosis (2). The *ERBB2* (HER2/neu) oncogene regulates cell proliferation, migration, differentiation, apoptosis and cell motility (3) through activation of the PI3K/AKT, STAT and the Ras/Raf signaling pathway (4). Trastuzumab, a humanized monoclonal antibody against the extracellular domain of HER2 significantly prolongs PFS and OS when added to chemotherapy in the metastatic setting (2). After failure of trastuzumab, the combination of capecitabine with lapatinib, a dual tyrosine kinase inhibitor of EGFR/ErbB1 and HER2, has been approved (5). Furthermore, the chemotherapy-free dual inhibition of HER2 with lapatinib plus trastuzumab yielded responses even in heavily pre-treated patients (6).

Anthracyclines showed a high efficacy in the treatment of HER2-positive breast cancer (7). Limitation of both drugs is cardiotoxicity, particularly when administered in combination (8). Through liposomal formulations, the cardiotoxicity of anthracyclines can be significantly reduced (9). Pegylated liposomal doxorubicin (PLD) showed high efficacy in phase II trials in patients with advanced breast cancer in combination with both, trastuzumab and vinorelbine (10, 11). As lapatinib appears to be less cardiotoxic than trastuzumab, its combination with PLD seems to be a good treatment option for HER2-positive MBC after previous therapy with cardiotoxic agents like conventional anthracyclines or trastuzumab. In addition, regarding the fact that one third of patients with HER2-positive breast cancer develop brain metastases, it may be of advantage that both agents can cross the blood brain barrier (BBB) (12, 13). In terms of a personalized cancer therapy, the establishment of prognostic and predictive markers is of great clinical importance. Therefore, we investigated the mutational status of several genes involved in tumor development and progression (*PIK3CA, PTEN, TP53, KRAS* and *BRAF*).

Patients and Methods

Patients. Patients with histologically confirmed, locally advanced, inoperable or metastatic breast cancer, with confirmed HER2 over-expression (IHC 3+ and/or FISH/SISH positive), were eligible for inclusion. At least one measurable lesion according to Response Evaluation Criteria in Solid Tumours (RECIST) Version 1.0 and documented disease progression after trastuzumab therapy (palliative or adjuvant) was required. Patients with severe cardiac disease and clinically apparent brain metastases were excluded from this study. This trial was conducted in accordance with Good Clinical Practice guidelines, all applicable local laws and the Declaration of Helsinki. The study protocol was approved by the local ethics committee. This trial was registered at ClinicalTrials.gov (NCT00903656) on 14 May 2009.

Study design and statistical assessments. For this open-label, multicenter- phase II trial a single-arm two-stage Green-Dahlberg design was applied (14), testing an overall response rate (CR or PR) of 10% *versus* 30% on a significance level of 0.05 with statistical power of 80%. The intention to treat (ITT) analysis population included all patients who received at least one dose of the study medication and was used for the analysis of efficacy. In a first step, a total of 20 patients was accrued and treated. The study was planned to be stopped if in this cohort less than 2 patients (10%) achieve a response. Otherwise, further 10 patients were planned to be included for a total of 30 patients. The regimen was concluded to be effective if seven or more responses out of 30 (23.3%) are observed at the end of the trial. The Kaplan-Meier methodology was used to estimate OS and PFS. The 95% confidence intervals for ORR were estimated according to a modified Wald method (15). All safety analyses were based on the safety population, which received at least one dose of the study medication and had at least one post-treatment safety assessment. To detect differences in quality of life (QoL), parametric paired Student`s t-tests or non-parametric Wilcoxon signed rank tests were used. Statistical significance was defined as p-value <0.05.

Table I. *Patients' characteristics (n=24).*

Characteristics	n (%)
Median age (years, range)	58 (40-78)
Average time since diagnosis (months, range)	52 (6-185)
HER2 status	
IHC 3+ (only)	22 (91)
FISH+ (with or without IHC)	2 (8)
ER/PgR status	
ER+ and/or PgR+	8 (33)
ER– and PgR–	16 (67)
Adjuvant treatment	
Anthracycline containing regimen	9 (37)
Taxane containing regimen	1 (4)
Anthracycline and taxane containing regimen	13 (56)
Endocrine therapy only	1 (4)
Trastuzumab containing regimen	10 (42)*
Radiotherapy	12 (50)
Palliative treatment	
No prior treatment for advanced disease	4 (16)
1 prior treatment line total	13 (56)
1 prior treatment line including trastuzumab	12 (50)
2 prior treatment lines total	2 (8)
2 prior treatment lines including trastuzumab	1 (4)
3 prior treatment lines total	4 (16)
3 prior treatment lines including trastuzumab	4 (16)
Metastatic site	
Non-visceral disease only (bone/skin/lymph nodes-soft tissue)	4 (16)
Visceral disease only	6 (25)
Non-visceral and visceral disease	14 (58)

*In part patients were treated before the approval of adjuvant trastuzumab. Also, spell out all abbreviated terms.

Treatment. Lapatinib was administered orally once daily at a dosage of 1,250 mg (supplied as 250 mg tablets) continuously until disease progression. PLD was administered intravenously at a dosage of 40 mg/m^2 every 4 weeks for a maximum of 6 cycles. The dosage of lapatinib was equal to the dosage used in combination with capecitabine in phase III trials (5). PLD was administered in a slightly reduced dosage according to an Austrian observational study (16). On the basis of adverse events (AEs) and laboratory abnormalities, dose modifications were performed.

Endpoints and assessments. The primary objective of this study was ORR (CR or PR), determined by radiologic evaluation in eight-weekly intervals according to RECIST Version 1.0. Secondary objectives were to determine the safety profile of this drug combination in terms of qualitative and quantitative toxicities, the occurrence of clinically apparent brain metastases, OS, PFS, clinical benefit rate (CBR) (CR, PR or stable disease for at least 24 weeks) and the QoL status using the EORTC QLQ-C30 standard questionnaire, respectively. Safety assessments were conducted in four-weekly intervals and AEs were MedDRA- (Medical Dictionary for Regulatory Activities) coded. Toxicity grading was done

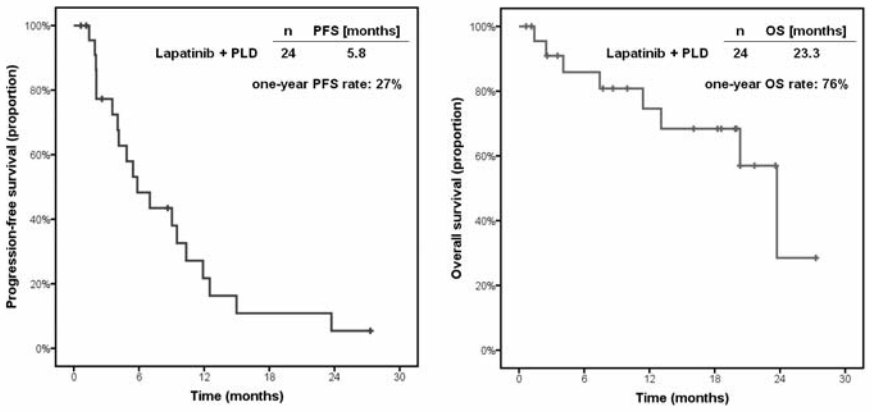

Figure 1. *Progression-free (PFS) and overall survival (OS).*

according to Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Translational research. In a supplementary scientific research program the frequencies of common mutations in the *KRAS* (Exon 2+3+4), *BRAF, PIK3CA* (Exon 9+20), *TP53* (Exon 5+6+7+8+9) and *PTEN* (Exon 5+7+8) gene were investigated. DNA was obtained from formalin-fixed and paraffin-embedded (FFPE) tumour samples and was subjected, on the one hand, for real time detection polymerase chain reaction (RTD-PCR) with primers specifically designed to amplify and detect cancer-related somatic mutations in human *BRAF* V600E and PIK3CA and, on the other hand, for sequence analysis of somatic mutations in human *PTEN, KRAS* and *TP53*, respectively (17-20).

Results

Efficacy. A total of 24 breast cancer patients from 7 Austrian medical Centers were included between July 2009 and August 2011 in the present study. Patients' characteristics are summarized in Table I. Among the first 20 patients included, 6 showed a PR (30%) and the second stage of the study was initiated. Unfortunately, the trial had to be stopped after 24 patients were included, because PLD was not available due to delivery problems by the manufacturer. Best overall response at the end of the trial was observed in 13 of 24 patients (54.2%), including one CR and 12 PRs. The ITT response rate of 54.2% (95% confidence interval (CI)=35%-73%) clearly allows rejecting the null hypothesis (ORR=10%, $p<0.001$). A further 6 patients achieved stable disease (SD) accounting for a CBR of 79%. Three patients (13%) had progressive disease (PD). For 2 patients no outcome data were available because of trial discontinuation (patient decision) prior to first staging. Median PFS was 5.8 months with a one-year PFS rate of 27%. Median OS was 23.3 months with a one-year overall survival rate of 76% (Figure 1). All deaths were directly related to breast cancer.

Table II. *Toxicities (n=24).*

	All grades, n (%)	Grade 3, n (%)	Grade 4, n (%)
Anaemia	8 (33%)	0	0
Thrombopenia	2 (8%)	0	0
Neutropenia	1 (4%)	0	0
Rash	12 (50%)	0	0
Mucositis	4 (17%)	0	0
Diarrhea	13 (54%)	2 (8%)	0
Nausea	7 (29%)	0	0
Vomiting	3 (12%)	0	0
Fatigue	7 (29%)	0	0
Decreased appetite	2 (8%)	0	0
Infection*	12 (50%)	2 (8%)	0
Neuropathy peripheral	2 (8%)	1 (4%)	0
Heart failure	0	0	0

*One patient died due to pneumonia (grade 5).

The most common cause of treatment discontinuation was disease progression, while 5 patients dropped out due to toxicity and 3 patients withdrew their informed consent. One patient died during study participation because of infectious pneumonia and another developed clinical apparent brain metastases during treatment.

Safety. A total of 192 AEs were reported. Most of they were of grade 1 (59%) or grade 2 (30%). Skin disorders were most frequent (33%), followed by gastrointestinal disorders (30%). No cardiac events were observed. Ten patients experienced at least 1 severe adverse event (SAE); 13 SAEs were reported in total. Eight of these SAEs were considered related to study treatment and none was classified as a Suspected Unexpected Severe Adverse Reaction (SUSAR). AEs are listed in Table II.

Translational research. Common mutations in the *KRAS, BRAF, PIK3CA, TP53* and the *PTEN* genes were analyzed in tumor samples from 19 participating patients. No mutation in the *KRAS* gene was found; *BRAF* was mutated in 3 samples (16%); *PTEN, PI3CA* and *TP53* in 1 tumour sample (5%) each, respectively.

Discussion

In the present trial we investigated the efficacy and safety of a combination of lapatinib and PLD in patients with HER2-positive advanced breast cancer progressing during or after trastuzumab therapy. To our knowledge, this is the first trial to study this specific drug combination. Our findings suggest that lapatinib plus PLD are active in this patient cohort with an ORR of 54%, a median PFS of 5.8 months and a one-year PFS rate of 27%. A median OS of almost 2 years (23.3 months) fortifies the effectiveness of this treatment option, especially because all, except 4, patients had visceral metastases (83%). Only 1 patient (4%) developed clinically-manifested brain metastases. During treatment with trastuzumab, a considerably higher rate of about 30% has been reported in the literature (21). In a recently presented phase III trial, no difference in the rate of central nervous system (CNS) metastases between lapatinib and trastuzumab was found when combined with capecitabine (3% *vs*. 5%) (22). Since both drugs under investigation cross the BBB, the low rate of CNS metastases observed might point to an additive or even synergistic effect of lapatinib and PLD in this regard. In addition, lapatinib, as well as PLD, were well-tolerated. Maximal toxicities of grade 3 were rare, with skin and gastrointestinal toxicity being the most common. No cardiac event was documented in our patient cohort during study treatment. This stands well in line with previous reports that both, lapatinib and PLD show less cardiotoxicity compared to trastuzumab and conventional anthracyclines, respectively (10, 11). In order to identify predictive and prognostic markers, the frequency of common mutations in several genes (*KRAS, BRAF, PIK3CA, TP53, PTEN*), involved in oncogenesis, were investigated in tumor samples from 19 participating patients. The formalin-fixed and paraffin-embedded (FFPE) tumor samples of the 5 remaining patients were not available. No mutation in the *KRAS* gene was found, *BRAF* was mutated in 3 samples (16%); *PTEN, PI3CA* and *TP53* in 1 tumour sample (5%), respectively. Mutation frequencies in our cohort differed from the frequencies described in the literature: *TP53*, for example, was found mutated in 5% of our patients only, while expected at a much higher rate of up to 75%, as reported in the literature (23). *BRAF* mutations, in contrast, with 16% were more frequent in our cohort than previously described in HER2-positive breast cancer (3%) (19). Also, the rate of *PIK3CA* and *PTEN* mutations, as well as *PTEN* loss, were unexpectedly low with 5% each *versus* 32-42% (*PIK3CA*) and 19-24 (*PTEN*), respectively (23). This discrepancy can be explained by the small number of samples in this trial or by the fact that frequencies described in the literature were originated from stage I-III tumours and not from stage IV tumours. The genetic profile of both might be different. During the performance of this study, trastuzumab emtansine (TDM-1), a HER2-targeting monoclonal antibody armed with the spindle toxine emtansine, showed high efficacy against trastuzumab- and lapatinib-resistant cases of metastatic breast cancer and was approved in February 2013 by the FDA and in November 2013 by the EMA (24). However, TDM-1 is not expected to enter the BBB and resistance inevitably occurs even with this novel drug. Therefore, additional drugs or drug combinations, as the one tested in our trial, are needed in order to transform HER2-positive breast cancer into a chronic disease.

Conclusion

In conclusion, lapatinib plus PLD has proven to be an active and safe treatment option in patients with HER2-positive advanced breast cancer. Both drugs have the ability to cross the BBB and show low cardiac toxicity, rendering this drug-combination suitable, especially for patients with cardiologic risk factors or brain metastases. However, in such patient sub-groups, further investigation of this combination is warranted.

Conflicts of Interest

M.A. Fridrik currently receives consultancy/advisory fees from Janssen; R. Greil has a consultant/advisory role for GlaxoSmithKline. All other authors declare that they have no competing interests.

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