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Cardiac safety of trastuzumab in combination with epirubicin and cyclophosphamide in women with metastatic breast cancer: results of a phase I trial

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Abstract

This prospective, parallel-group, dose-escalation study evaluated the cardiac safety of trastuzumab (Herceptin®) plus epirubicin/cyclophosphamide (EC) in women with human epidermal growth factor receptor-2 (HER2)-positive metastatic breast cancer (MBC) and determined an epirubicin dose for further evaluation. HER2-positive patients received standard-dose trastuzumab plus epirubicin (60 or 90 mg/m^2)/cyclophosphamide (600 mg/m^2) 3-weekly (EC60 + H, $n = 26$; EC90 + H, $n = 25$), for four to six cycles; 23 HER2-negative patients received EC alone (90/600 mg/m^2) 3-weekly for six cycles (EC90). All patients underwent thorough cardiac evaluation. Two EC90 + H-treated patients experienced symptomatic congestive heart failure 4.5 and 6 months after the end of chemotherapy. One EC60 + H-treated patient experienced an asymptomatic decrease in left ventricular ejection fraction (LVEF) to $<50\%$ 6 months after the end of chemotherapy. No such events occurred in control patients. Asymptomatic LVEF decreases of $>10\%$ points were detected in 12 (48%), 14 (56%) and 5 (24%) patients treated with EC60 + H, EC90 + H, and EC90. Objective response rates with EC60 + H and EC90 + H were $>60\%$, and 26% for EC90 alone. These results indicate that trastuzumab may be combined with EC with manageable cardiotoxicity and promising efficacy.

Keywords: Trastuzumab; Metastatic breast cancer; HER2; Anthracyclines; Epirubicin; Cyclophosphamide; Cardiotoxicity

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1. Introduction

The gene encoding the human epidermal growth factor receptor-2 (HER2, also know as c-erbB-2 or neu) is amplified and the protein overexpressed in 20–25% of breast cancers [1–3]. This abnormality is associated with aggressive disease and poor prognosis [4–6]. In addition to its prognostic significance, HER2 positivity may have predictive value for the likelihood of response to cyclophosphamide, methotrexate and fluorouracil, hormonal therapy, anthracyclines and taxanes [7–9].

The occurrence of HER2 amplification early in the course of breast cancer [10] and evidence that it causes malignant transformation [8] led to the development of the humanised anti-HER2 monoclonal antibody trastuzumab (Herceptin®) [11]. Trastuzumab monotherapy is active and well tolerated in women with HER2-positive metastatic breast cancer (MBC), both as first-line therapy [12] and in patients whose disease has progressed after receiving chemotherapy for MBC [13]. Treatment with trastuzumab has been shown to prolong survival from a median of 20.3–25.1 months when used in combination with chemotherapy. Furthermore, it increases the objective response rate (from 32% to 50%) and extends time to progression (4.6–7.4 months) when used first line in combination with an anthracycline plus cyclophosphamide (AC) or paclitaxel in women with HER2-positive MBC [14]. Response rate and survival duration (56% and 26.8 months, respectively) were greatest in patients treated with trastuzumab plus AC, most of whom received doxorubicin.

Based on a retrospective analysis, the combination of trastuzumab with AC was associated with a greater risk of cardiotoxicity than AC alone in this trial (27% versus 8% of patients, respectively) [14,15]. Cardiotoxicity was manifest as decreases in left ventricular ejection fraction (LVEF), with or without signs and symptoms of congestive heart failure (CHF). Cardiotoxicity was usually reversible using standard medication, and often with continued trastuzumab therapy. Prior or concomitant anthracycline exposure has been identified as the most significant risk factor for cardiotoxicity in patients receiving trastuzumab [15]. Therefore, the combination of trastuzumab with anthracyclines is not currently approved for use outside clinical trials.

Anthracyclines are very active in the treatment of breast cancer and preclinical data show that trastuzumab has additive, or even synergistic, activity in combination with a number of chemotherapeutic agents, including anthracyclines [16]. Clinical data also showed that the addition of trastuzumab to AC produced an overall survival benefit even when cardiac events are taken into account, and that most deaths were due to progressive breast cancer and not cardiac failure [17]. Patients who received doxorubicin and epirubicin in this trial were not differentiated, although it is known that the majority of patients received doxorubicin rather than epirubicin. Therefore, the combination of trastuzumab and anthracyclines, and particularly the less cardiotoxic anthracycline, epirubicin, is worthy of further investigation. Trastuzumab plus anthracyclines may potentially have a role as first-, second-, or third-line therapy, following prior adjuvant treatment with taxanes, or even in patients who relapse after a long disease-free interval following anthracycline treatment. To this end, studies exploring the use of trastuzumab with anthracyclines other than doxorubicin are in progress [18].

Epirubicin is active in primary and MBC, with similar efficacy to doxorubicin but less cardiotoxicity [19]. The aim of the present phase I/II study was to evaluate the cardiac safety of trastuzumab plus epirubicin/cyclophosphamide (EC) in patients with HER2-positive disease and to compare it with that of EC alone in a parallel cohort of patients with HER2-negative breast cancer. Results of the phase I part of this study are presented here.

2. Methods

2.1. Study design

This is the prospective, multicentre, open-label, phase I, parallel-group, dose-escalation part of a phase I/II study conducted in 25 centres in Germany. The primary objective was to evaluate the cardiac safety of EC plus trastuzumab in women with HER2-positive MBC and compare it to that in women with HER2-negative MBC receiving EC alone. The secondary objective was to evaluate efficacy.

During this dose-escalation part, 25 HER2-positive patients were scheduled to be recruited to receive epirubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2, trastuzumab (EC60 + H). If cardiotoxicity was acceptable, dose of epirubicin would be escalated to 90 mg/m^2 (EC90 + H). 25 patients would then be recruited at this dose and 25 HER2-negative patients would be recruited to receive chemotherapy alone as a comparator group (EC90). All patients underwent cardiac assessment according to a predefined schedule (see below).

The decision whether to escalate from EC60 + H to EC90 + H or to terminate the study was based on the incidence of dose-limiting cardiotoxicity (DLC) observed during and up to 3 weeks after six cycles of chemotherapy plus trastuzumab. DLC was defined as: an absolute decrease in LVEF of $>10\%$ points from the value at screening and to $<50\%$; clinical signs of CHF (NYHA functional class I–IV); severe arrhythmia requiring therapy; acute coronary syndrome or acute myocardial infarction requiring therapy; or need for cardiopulmonary resuscitation. In case of no or one

cardiac event, the study could continue with escalation to EC90 + H. If 2–4 cardiac events occurred, the Steering Committee would decide whether to escalate the dose to EC90 + H. The occurrence of five or more cardiac events would lead to trial termination.

After treating 25 patients each with six cycles of EC90 with or without trastuzumab, cardiac safety would again be assessed 3 weeks after the end of chemotherapy and compared to the EC60 + H arm. The Steering Committee would then base its recommendation for the subsequent phase II part of the study on the number of cardiac events seen with EC90 + H. EC60 + H would be used in the event of five or more cardiac events with EC90 + H; if two to four events occurred, the Steering Committee would decide on which dose to use; and no or one cardiac event would lead to full recruitment at EC90 + H (phase II: additional 75 patients to both the EC90 + H and EC90-alone groups, resulting in a total of 100 patients in each). Throughout the dose-escalating part of this study, cardiac events were reviewed continuously by the Steering Committee in order to determine whether they met the criteria for DLC, and to decide whether the criteria for dose reduction (or trial termination) had been met or whether any modification to the protocol was required. In addition, the steering committee reviewed LVEF data monthly.

The protocol was approved by the ethics committees of each participating centre and was conducted in accordance with the principles of the revised Declaration of Helsinki (1996) and international Good Clinical Practice (GCP) guidelines. All patients gave written informed consent before inclusion in the study.

2.2. Patients

Women aged 18–70 years with histologically confirmed, untreated recurrent or MBC, ECOG performance status <2 and life expectancy $\geqslant 3$ months were eligible. Knowledge of HER2 status was mandatory.

Exclusion criteria were: prior anti-HER2 treatment, cytotoxic chemotherapy for MBC, prior adjuvant anthracycline-containing chemotherapy or high-dose chemotherapy with peripheral stem-cell transplantation; bone or central nervous metastases as the only site of metastasis; history of other malignancy; serum creatinine $>1.5\times$ upper limit of normal (ULN); bilirubin $>1.5\times$ ULN; transaminases or alkaline phosphatase $>2.5\times$ ULN or $>5.0\times$ ULN in case of liver or bone metastases; serum calcium $\geqslant 12.0$ mg/dl (3.0 mmol/l); pregnancy or lack of a reliable appropriate contraceptive method in women of child-bearing potential; previous participation in this study or in another study in the previous 4 weeks; or any medical condition likely to interfere with the conduct of the study. In addition, specific cardiovascular exclusion criteria were: LVEF $<55\%$ determined by two-dimensional echocardiography at rest; prior treatment with cardiotoxic agents; past or present coronary heart disease, valvular disease requiring treatment, cardiomyopathy, or acute myocarditis; CHF; end-diastolic left ventricular diameter >56 mm determined by M-mode echocardiography at rest; arrhythmias requiring treatment; poorly controlled arterial hypertension; or prior mediastinal irradiation.

2.3. HER2 testing

HER2 status was determined using archived primary tumour samples and a standard semiquantitative IHC test (DAKO HercepTest™) or FISH analysis (Vysis or Ventana). Inclusion of patients could be based on either local or central laboratory testing. Patients with tumours that were IHC 3+ and/or FISH positive were eligible for the trastuzumab-containing treatment arms.

2.4. Treatment

On day 1, intravenous trastuzumab, epirubicin and cyclophosphamide were administered according to standard prescribing information. Trastuzumab was administered as a 4 mg/kg loading dose followed by 2 mg/kg weekly until disease progression. Trastuzumab was withheld in the event of drug-related grade 3 or 4 non-haematological toxicity until recovery to grade 2 or better. In the event of recurrence of grade 3 or 4 non-haematological toxicity, trastuzumab was to be discontinued. Trastuzumab treatment was continued in the presence of haematological toxicity.

EC was administered every 3 weeks for six cycles at dose level I (epirubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2) and for four to six cycles at dose level II (epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2). Epirubicin was administered intravenously over 30 min and was followed by intravenous cyclophosphamide over 30 min. The first dose of chemotherapy was administered 3 h after trastuzumab; subsequent doses were administered immediately after trastuzumab if the initial dose was well tolerated. Patients were scheduled to receive all cycles of chemotherapy at the same dosage. Treatment could be postponed for a maximum of 1 week only in the event of severe haematological or non-haematological toxicity. If there was no improvement in toxicity during this period, chemotherapy was to be discontinued.

Palliative and supportive care for disease- and treatment-related symptoms (e.g. analgesics, paracentesis, premedication and hydration) was offered to all patients when indicated, and palliative radiotherapy was permitted if it did not compromise evaluation of disease response. Any drug except antineoplastic drugs/agents, dexrazoxane or prophylactic G-CSF could be administered concomitantly.

2.5. Assessment

Cardiac function and adverse events were assessed using National Cancer Institute Common Toxicity Criteria (NCI-CTC) at screening, weeks 1, 4, 7, 10, 13 and 16 during combination therapy, post-chemotherapy at week 19, and 12-weekly thereafter until week 103 or disease progression. Serious adverse events were reported immediately.

LVEF was measured by two-dimensional echocardiography, examinations were recorded on videotape and the four-chamber view was used to assess ejection fraction (EF) in all patients. Ventricular volumes were measured by manual planimetry. The end-diastolic left ventricular (LF) volume was measured at the Q wave of the QRS complex. For the end-systolic LV volume, the smallest detectable volume was used. EF was calculated using the formula:

EF = (end-diastolic LV volume − end-systolic LV volume)/end-diastolic LV volume

Serum cardiac marker concentrations (N-terminal brain natriuretic peptide and cardiac troponin-T) were measured over time to identify any correlation between changes of these markers and LVEF changes or symptoms of CHF. Results of this sub-study will be presented separately.

Response was assessed every 6 weeks during the treatment period and every 12 weeks during follow-up using World Health Organisation (WHO) criteria. Secondary efficacy parameters included time to progression and overall response rate (complete remission (CR) plus partial remission (PR)), defined according to WHO criteria for progression and remission.

2.6. Statistical considerations

The aim of this part of the study was to identify an anthracycline-containing combination regimen associated with an acceptable rate of DLC using predefined criteria. The EC + H combination would be considered tolerable if the observed rate of DLC was $<10\%$ and the number of patients was large enough to ensure that a true rate of $\geqslant 15\%$ could be excluded statistically with 90% confidence. A cohort of patients treated with EC alone was included to estimate the difference in predefined cardiotoxicity between the two treatment regimens and the contribution of trastuzumab to DLC. To detect a difference in DLC of 5% (DLC with EC of about 5% versus $\geqslant 10\%$ with EC + H) would require a very large sample size. For this reason, the analysis is descriptive in nature. In the first stage, a cohort of 25 patients was selected on the basis that the dose level could be accepted as sufficiently tolerable if fewer than two cases of DLC were observed because the one-sided 90% confidence interval (CI) would be $<15\%$. In this instance, the decision to escalate to dose level II would be justified. If the same situation occurred at dose level II, 75 additional patients for each arm were to be included in the second stage of the study.

3. Results

3.1. Patient demographics

75 patients were enrolled between June 2000 and June 2002. A total of 26 patients were treated with EC60 + H (dose level I). 25 patients were then enrolled in the second cohort and treated with EC90 + H (dose level II). 24 patients with HER2-negative disease were recruited, one of whom was excluded prior to treatment due to violation of selection criteria (abnormal baseline ECG). Patients' baseline characteristics are shown in Table 1. Apart from the differences in HER2 status as defined in the protocol, the three cohorts did not differ

Table 1
Patient characteristics at baseline

	EC60 + H ($n=26$)	EC90 + H ($n=25$)	EC alone ($n=23$)[a]
Mean age, years (range)	54 (31–69)	57.6 (41–69)	54.5 (38–67)
HER2 status, *n* (%)			
IHC 2 + /FISH +	1 (4)	3 (12)	–
IHC 3 +	25 (96)	22 (88)	–
ER positive, *n* (%)	13 (50)	12 (48)	18 (78)
ECOG performance status, *n* (%)			
0	20 (77)	19 (76)	16 (70)
1	6 (23)	6 (24)	7 (30)
Disease stage at initial diagnosis			
I	3 (12)	1 (4)	5 (22)
II	7 (27)	9 (36)	10 (44)
III	3 (12)	2 (8)	–
IV	12 (46)	13 (52)	8 (35)
Not known	1 (4)	–	–
Median no. of metastatic sites at enrolment (range)	2 (1–7)	3 (1–7)	2 (1–5)
Disease site			
Lung	14 (54)	10 (40)	11 (48)
Liver	16 (62)	15 (60)	14 (61)
Bone	8 (31)	11 (44)	11 (48)
Soft tissue	8 (31)	9 (39)	13 (52)
Prior therapy, *n* (%)			
Adjuvant chemotherapy	8 (31)	7 (28)	10 (44)
Hormonal therapy	8 (31)	7 (28)	9 (39)
Radiotherapy	11 (42)	9 (36)	11 (48)

[a] Epirubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2, trastuzumab (EC60 + H); epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2, trastuzumab (EC90 + H); epirubicin 90 mg/m^2 (EC90) alone.

significantly in terms of prior treatment or cardiac risk factors.

Exposure to study drugs was similar in all three cohorts (median number of cycles of epirubicin and cyclophosphamide in all three cohorts: 6). Patient withdrawal is shown in Fig. 1.

3.2. Cardiac safety

Median LVEF at baseline in the EC60 + H, EC90 + H and EC-alone cohorts was 70% (range: 57–82%), 71% (60–90%) and 70% (58–79%), respectively. Changes in LVEF from baseline over six cycles of therapy are shown in Fig. 2 and Table 2. Overall asymptomatic falls in LVEF of >10% points during the whole observation period were detected in 12 patients (48%) treated with EC60 + H, 14 patients (56%) treated with EC90 + H and 5 patients (24%) treated with EC90 alone. Decreases of >10% and to <50% occurred in 3 patients only during continuation of treatment with trastuzumab monotherapy (see below).

Eight cardiac adverse events, none of which fulfilled the protocol-defined criteria for cardiotoxicity, were reported in 5 patients during treatment with EC60 + H. These included: arrhythmia (2 patients); atrioventricular block (1); hypokinesia (1); swelling of the lower limb (2); palpitations (1); and supraventricular tachycardia (1). In the EC90 + H cohort, one cardiac event (transient absolute arrhythmia) was reported and considered related to trastuzumab, but did not fulfil the protocol-defined criteria for DLC according to the Steering Committee. Three protocol-defined cardiotoxic events occurred in 3 patients treated with EC + H (1 EC60 + H, 2 EC90 + H), with LVEF decreasing by >10% points to <50%, 5–6 months after the end of chemotherapy. The events were judged to be related to study treatment in all 3 women and are summarised below; LVEF measurements for these 3 patients are shown in Table 3.

A 61-year-old patient, who had undergone left thoracic irradiation for stage IIA breast cancer ($T_2N_0M_0$) 2 years prior to study entry but had no cardiac history, received six cycles of EC60 + H. Her LVEF was 73% at baseline and 58% on completion of chemotherapy. Six months after completing chemotherapy, during treatment with trastuzumab alone, her LVEF decreased to 44% without symptoms of heart failure, and trastuzu-

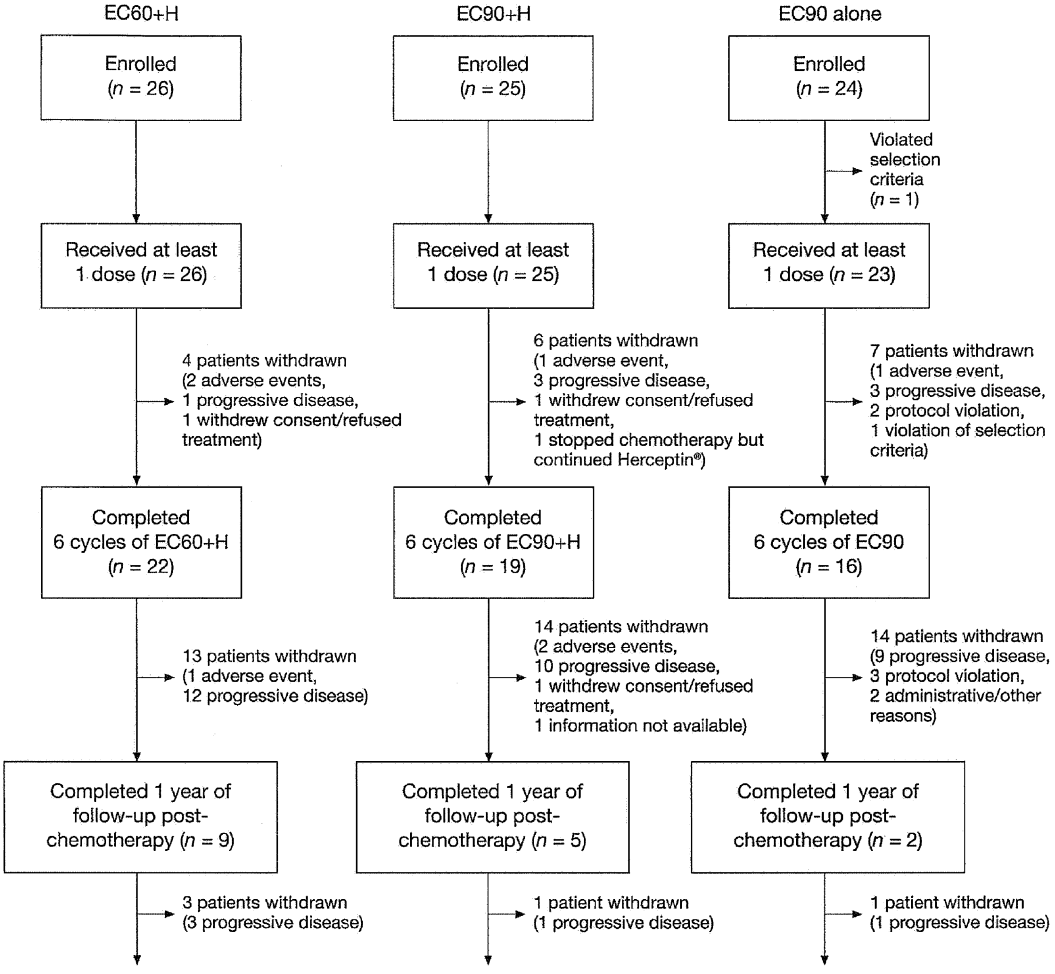

Fig. 1. Patient disposition.

Table 2
Changes in left ventricular ejection fraction from baseline over the period of assessment, start of therapy (week 1) until 3 weeks after cycle 6 (week 19)

Regimen	Visit	Number of patients (%)			
		Increase or no change	Decrease $\leqslant 10\%$	Decrease $>10\%$	Decrease $>10\%$ and to $<50\%$
EC60 + H[a]	After cycle 3 ($n=22$)	10 (45%)	7 (32%)	5 (23%)	0
	After cycle 6 ($n=21$)	8 (38%)	10 (48%)	3 (14%)	0
	Overall worst value ($n=25$)	2 (8%)	11 (44%)	12 (48%)	1 event*
EC90 + H	After cycle 3 (n = 25)	9 (36%)	13 (52%)	3 (12%)	0
	After cycle 6 ($n=18$)	4 (22%)	11 (61%)	3 (17%)	0
	Overall worst value ($n=25$)	3 (12%)	8 (32%)	14 (56%)	2 events*
EC90 alone	After cycle 3 ($n=16$)	10 (63%)	5 (31%)	1 (6%)	0
	After cycle 6 ($n=16$)	8 (50%)	7 (44%)	1 (6%)	0
	Overall worst value ($n=21$)	3 (14%)	13 (62%)	5 (24%)	0

*Occurred during follow-up.
[a] Abbreviations as in Table 1.

mab was stopped. Her LV dysfunction had not improved after one month. At this time, echocardiography revealed grade I mitral valve insufficiency, grade II tricuspid valve insufficiency and a reduction in global LV function, plus mild pulmonary hypertension. At most recent follow-up, 8 months after diagnosis of these abnormalities, echocardiography showed normal global LV function, although the other abnormalities persisted. No therapeutic measures were taken in response to these events.

A 57-year-old patient with a history of hypertension and peripheral oedema was treated with six cycles of EC90 + H 11 years after treatment of primary breast cancer (stage IIA; $T_0N_1M_0$). Her LVEF was 63% at baseline and 77% on completion of chemotherapy. Five months after completing chemotherapy, during treatment with trastuzumab alone, she presented with progressive dyspnoea and orthopnoea, and CHF was diagnosed (NYHA grade III). Her LVEF was found to be 44%, and echocardiography revealed pulmonary congestion, hypokinesia and mitral regurgitation. Trastuzumab therapy was stopped and she was treated with an angiotensin-converting enzyme inhibitor and diuretics. One month later, her cardiac failure was reported improved but not resolved.

Finally, a 64-year-old woman, with stage I breast cancer ($T_1N_0M_0$) diagnosed 9 years prior to study entry, completed treatment with EC90 + H and continued to receive trastuzumab monotherapy. She had no history of cardiovascular disease and no cardiac risk factors at study entry, although she had received irradiation to the right thoracic wall, regional lymph nodes and the site of local recurrence. Her LVEF was 82% at baseline and 73% on completion of EC90 + H. NYHA grade II CHF was diagnosed 6 months after completing chemotherapy, when her LVEF was 60%. No treatment was given and trastuzumab therapy was continued. Her LVEF was found to be 49% 3 months after the

Table 3
Left ventricular ejection fraction (LVEF) measurements in the three women who developed protocol-defined cardiac events

Time of LVEF measurement (weeks)	LVEF (%)		
	Patient 1 (age 61 years; received EC60 + H)[a]	Patient 2 (age 57 years; received EC90 + H)	Patient 3 (age 64 years; received EC90 + H)
0 (baseline)	73	63	82
3	–	–	67
4	–	55	82
6	–	60	–
9	–	68	67
12	52	62	81
15	67	74	75
18 (end of therapy)	58	77	73
30	–	70	75
35	55	44*	-
42	44*	–	60*
55	–	–	49

*Cardiac event diagnosed.
[a] Abbreviations as in Table 1.

Table 4
Side-effects (other than cardiac events) observed in >10% of patients in any patient group

Side effect	% Of patients		
	EC60 + H[a]	EC90 + H	EC90 alone
Alopecia	35	40	35
Nausea	38	28	22
Vomiting	19	12	13
Arthralgia	23	12	4
Fatigue	8	20	13
Cough	12	12	9
Constipation	12	12	4
Diarrhoea	12	12	4
Headache	12	8	9
Nasopharyngitis	12	4	13
Mucositis/stomatitis	12	20	4

[a] Abbreviations as in Table 1.

diagnosis of CHF. Trastuzumab was stopped and cardiovascular therapy consisting of hydrochlorothiazide, metoprolol and symptom-adjusted ramipril was started. Pulmonary metastases were also diagnosed at this time and the patient withdrawn from the study due to disease progression.

3.3. Other safety data

The most common non-cardiac and non-haematological adverse events were alopecia, nausea, vomiting and arthralgia (Table 4). Such events tended to occur at a similar incidence in all three patient groups, although a firm assessment cannot be made due to the difference in duration of observation for the three patient groups. The incidence of haematological toxicity was within the expected range and similar in all three groups.

3.4. Efficacy

The numbers of patients who could be evaluated for efficacy in the EC60 + H, EC90 + H and EC90-alone cohorts were 25, 25 and 23, respectively. The overall response rates for EC60 + H and EC90 + H were >60% (62% and 64%, respectively), and 26% with EC90 alone.

4. Discussion

The results of this study demonstrate that combination therapy with weekly trastuzumab plus 3-weekly EC does not produce an unacceptably high incidence of cardiotoxicity based on the protocol-defined criteria for this trial. In the 2 patients treated with EC90 + H who experienced symptomatic CHF and the patient treated with EC60 + H who experienced an asymptomatic decline in LVEF to <50%, events which met the pre-defined criteria for DLC, the events occurred at least 5 months after cessation of chemotherapy, during treatment with trastuzumab alone. Two of these patients had received prior radiotherapy to the thorax, which is a possible risk factor for trastuzumab-related cardiotoxicity [20]. It is not possible to draw any conclusions regarding the possible impact of radiotherapy on trastuzumab-associated cardiotoxicity based on this observation, but previous analyses have not identified a correlation between prior radiotherapy and trastuzumab-associated cardiotoxicity [15,21].

The majority of patients with HER2-negative disease were lost to cardiac follow-up before the time when cardiac events were observed in the trastuzumab-containing arms, i.e. 4–6 months after end of chemotherapy. For this reason, in the phase II part of this trial all patients will be followed for 2 years regardless of response to study treatment.

All patients in this trial had close cardiac monitoring with LVEF assessments every 3 weeks during chemotherapy. Asymptomatic reductions in LVEF were observed in all three patient groups and changes were more pronounced in the trastuzumab-containing treatment arms (Table 2). The clinical significance of these reductions is difficult to assess because intrapatient variations in LVEF over time were common and the open-label study design may have led to observation bias. It is noteworthy that asymptomatic reductions in LVEF do not always progress to CHF in patients with MBC, and that CHF may not be preceded by an asymptomatic reduction in LVEF, as was observed in one patient in this study.

The observation of cardiotoxicity in this trial was not unexpected, as a retrospective review of available data from 1219 patients in seven clinical trials has indicated that the incidence of cardiotoxicity is increased when trastuzumab is combined with anthracyclines, which are also associated with cardiotoxicity [15,22]. The potential mechanism for trastuzumab-associated cardiotoxicity remains unclear.

The efficacy results for treatment with trastuzumab plus EC are promising. The response rates for EC60 + H and EC90 + H, at 62% and 64%, respectively, are similar to those reported for patients treated with trastuzumab plus AC (predominantly doxorubicin), which was superior to AC or paclitaxel in patients with HER2-positive disease [14]. Time to progression and overall survival at 1 and 2 years are still under evaluation. In contrast, preliminary data for the control group appear to indicate that the response to EC alone in patients with HER2-negative disease (26%) is inferior to the trastuzumab-containing regimen in patients with HER2-positive disease. This observed response rate is comparable to the rate of 33.8% reported by Konecny and colleagues for patients with HER2-negative tumours treated with EC60 [23], but lower than that in

unselected populations treated with EC60 (response rate 41%) [24] or EC75 (56%) [25]. It is also possible that HER2 status influences the outcome of anthracycline therapy, with HER2-positive tumours responding better to anthracyclines than HER2-negative tumours [9]. Di Leo and colleagues have suggested that amplification of the gene encoding topoisomerase-IIα, which is the target for anthracyclines such as epirubicin and is often coamplified with HER2, is responsible for this increased sensitivity [26]. Others have shown that both

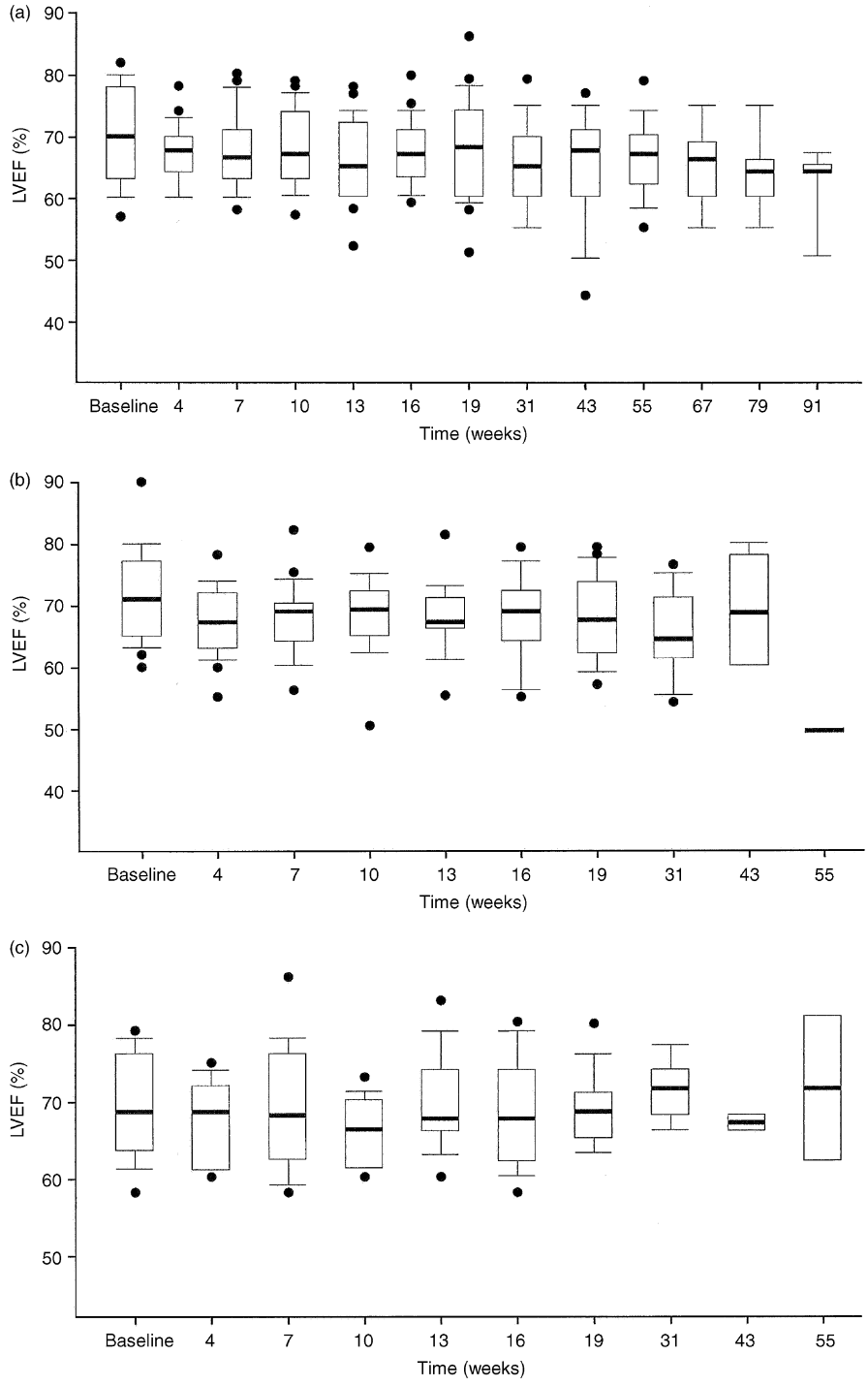

Fig. 2. Left ventricular ejection fraction during six cycles of epirubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2, trastuzumab (EC60 + H) ($n = 26$) (a); epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2, trastuzumab (EC90 + H) ($n = 25$) (b); and epirubicin 90 mg/m^2 (EC90) alone ($n = 23$) (c). The lower and upper limits of each box indicate the 25th and 75th percentiles, and the bars show the 10th and 90th percentiles. Closed circles indicate outliers.

HER2-amplified, topoisomerase-IIα-amplified and HER2-amplified, topoisomerase-IIα-normal breast tumours also respond better to anthracycline therapy than do HER2-negative, topoisomerase-IIα-normal tumours [27]. The observation that response was poor in patients with HER2-negative disease treated with EC alone needs to be confirmed by evaluation of a larger group of patients, and will be performed in the expanded phase of the trial.

The combination of trastuzumab plus EC has shown promising efficacy and tolerability, regardless of epirubicin dose. Based on this, the trial will be expanded to investigate further the safety and efficacy of this combination. This part of the trial will involve three arms: two in which women with HER2-positive MBC will receive trastuzumab-containing treatment (EC60 + H; EC90 + H) and one in which women with HER2-negative disease will receive EC90 alone. In addition, based on the observation that LVEF measurements were very variable, central review of echocardiograms has been incorporated into the next stage of the trial to try to improve the consistency of LVEF evaluation and the primary endpoint for the phase II study is being revised to focus on symptomatic events. These appear to occur 4–6 months after end of chemotherapy, and therefore all patients will be followed for 2 years.

In conclusion, combination therapy with trastuzumab plus an anthracycline and cyclophosphamide is highly effective in the treatment of MBC and warrants further study. Furthermore, these results indicate that it may be feasible to combine trastuzumab with the less cardiotoxic anthracycline, epirubicin, without the high risk of cardiotoxicity seen during treatment with trastuzumab plus doxorubicin (overall 27%; NYHA class 3 and 4, 16%) [14].

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