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ORIGINAL ARTICLE

Trastuzumab and gemcitabine as salvage therapy in heavily pre-treated patients with metastatic breast cancer

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Abstract
Purpose In Her2-postive metastatic breast carcinoma, first-line trastuzumab-based therapy is well established; many centres continue antibody treatment beyond disease progression. In this trial, we evaluated the efficacy and safety of gemcitabine and trastuzumab after earlier exposure to anthracyclines, docetaxel and/or vinorelbine, and trastuzumab.
Methods Twenty-nine consecutive patients were included as eligible. Patients received gemcitabine at a dose of 1,250 mg/m² on day one and eight, every 21 days. Trastuzumab was administered in three-week cycles. Clinical benefit rate (CBR; CR + PR + SD $\geq$ 6 months) was defined as primary endpoint.
Results As of July 2007, all patients are evaluable for toxicity, and 26 for response. Earlier therapies consisted of trastuzumab (100%), anthracyclines (100%), vinorelbine (96.6%), docetaxel (72.4%), and capecitabine (72.4%). 19.2% of patients experienced PR, and SD $\geq$ 6 months was observed in a further 26.9%, resulting in a CBR of 46.2%. Time to progression was median 3 months, and overall survival 17 months. Neutropenia (20.7%), thrombocytopenia (13.8%), and nausea (3.4%) were the only treatment-related adverse events that occurred with grade 3 or 4 intensity. Four patients (13.8%) developed brain metastases while on therapy.
Conclusions While CBR was low when compared to trastuzumab-based first-line therapy, it is higher than what would be expected from gemcitabine monotherapy in a similar setting. Together with the favourable toxicity profile, this regimen appears to be a safe and potentially effective salvage therapy option in a heavily pre-treated population.

Keywords Advanced breast cancer · Gemcitabine · Her2-positive disease · Palliative chemotherapy · Trastuzumab

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Background

A decrease in breast cancer mortality was reported in most western countries in recent years. This development is usually attributed to screening programs, reduced use of hormone replacement therapy, and advances in systemic therapy [1–4]. Still, even in stage I and II breast cancer, up to one third of patients are expected to experience disease recurrence [5]. Although effective treatment options are also available in advanced disease, metastatic breast cancer remains an incurable disease, with median survival ranging from 24 to 30 months [2, 3]. Therefore the primary aim of therapy is palliative—to improve quality, and perhaps length of life with a minimum of treatment related side effects.

Her2-positive breast cancer is associated with high recurrence rates and poor outcome [6–9]. The advent of trastuzumab (RhMab45D), a monoclonal humanized antibody directed against the extracellular domain of Her2 (human EGFR related 2), was a major breakthrough. Different mechanisms of action have been described: Inhibition of downstream signalling pathways, blockade of cleavage of the extracellular domain, internalization and degradation of the Her2 receptor protein, decrease of cyclin dependent kinase 2 (CDK2) activity via p27 induction, and inhibition of DNA repair. Antibody dependent cellular cytotoxicity (ADCC) apparently also plays a role [10, 11].

Best outcome in terms of overall survival is achieved when the antibody is administered upfront, i.e. from detection of metastatic disease [12, 13]. On the other hand, there is no data available from prospective, randomized phase III trials concerning the continuation of trastuzumab beyond progression. Still, treatment in multiple lines is common clinical practice [14]. This might change, as a recently published randomized phase III trial comparing the combination of capecitabine with lapatinib, a dual-tyrosine kinase inhibitor targeting tyrosine kinase domains of EGFR and Her2, with capecitabine alone following trastuzumab failure reported superior outcomes in the combination group [15]. Still, data from retrospective analyses or phase II studies suggest a potential benefit from ongoing trastuzumab treatment in a subgroup of patients [16–19]. This subgroup however cannot be defined prospectively, as mechanisms of resistance are poorly understood [11].

The combination of trastuzumab and taxanes is the best established first-line treatment option in Her2-positive disease, with vinorelbine being a possible alternative. When however trastuzumab is administered in multiple lines, no standard of care exists. Doxorubicine and trastuzumab is clearly effective, but an increased rate of cardiac events was observed; platinum derivatives might also cause major side effects. Capecitabine, while well established in Her2-negative breast cancer, was until recently not used in combination with trastuzumab due to a suspected antagonistic effect of trastuzumab when combined with capecitabine´s parent substance, 5-fluorouracil (5-FU) [20]. Newer clinical data, however, suggest that this is might not be true in vivo [19, 21].

Gemcitabine (2′,2′-difluorodeoxycytidine) is a cytotoxic agent with proven activity in a number of human cancers [22]. The substance is a fluorine-substituted cytarabine (Ara-C) analogue, which is one of the most effective drugs available in the treatment of certain blood malignancies [23], while activity of Ara-C in solid tumours is low [24]. Like Ara-C, gemcitabine is a prodrug necessitating intracellular activation to its active form, gemcitabine diphosphate and triphosphate. In contrast to Ara-C however, membrane permeability and enzyme affinity is enhanced, as well as duration of intracellular retention, causing prolonged inhibition of DNA synthesis [24, 25]. These factors explain the activity of the drug in solid cancers like NSCLC, pancreatic cancer, and breast carcinoma.

In metastatic breast cancer, gemcitabine monotherapy yields response rates in the range of 14–37% [26–28], while in combination with taxanes (with or without anthracyclines) response rates of 32–62% are reported [29–31]. Notably, the combination of docetaxel and gemcitabine appears to be as effective a first line therapy as the combination of docetaxel plus capecitabine, while non-haematological toxicity is decreased [29, 31, 32]. In general, the substance appears to have a favourable toxicity profile, which makes it an interesting option a heavily pre-treated population.

We initiated this study to evaluate the potential activity of the combination of trastuzumab and gemcitabine in a population pre-treated with trastuzumab, anthracyclines, taxanes, vinorelbine, and capecitabine.

Patients and methods

All patient data were collected at the First Department of Medicine and Cancer Centre, Clinical Division of Oncology, at the Medical University of Vienna, Vienna, Austria. All consecutive patients with Her2-positive metastatic breast cancer were included as eligible for gemcitabine plus trastuzumab. Treatment was performed in accordance with the ethical regulations of the Medical University of Vienna.

Patients

Twenty-nine consecutive patients were included from December 2004 until December 2006, and followed prospectively; all are currently evaluable for toxicity and twenty-six for response. Data were analyzed as of July 2007. All patients were diagnosed with histologically confirmed metastatic breast cancer. Inclusion criteria were as follows: At least one measurable lesion, Karnofsky performance score $\geq$70, life expectancy of >3 months, adequate haematological parameters as defined by WBC count $\geq$ 3,500 μl^{-1}, platelet count $\geq$ 100,000 μl^{-1}, haemoglobin levels >9 g/dl, adequate hepatic (serum bilirubin < 2.0 mg/dl), and renal (serum creatinine < 1.5 mg/dl) functions, and informed consent. Patients with controlled metastatic disease to the brain (after whole brain radiotherapy (WBRT), neurosurgical resection and/or stereotactic radiosurgery of one to three metastases) were also eligible. WBRT was applied at 6 MV LINAC (linear accelerator) by lateral opposed fields. Total dose was 30 Gy, applied in ten fractions within 2 weeks.

In eligible patients, earlier exposure to an anthracycline for early or advanced disease, and pre-treatment with an anti-microtubule agent (docetaxel or vinorelbine) and trastuzumab for metastatic disease was mandatory. Prior capecitabine exposure was allowed. Baseline staging was performed with CT-scans of the chest and the abdomen, bone scan, mammography, echocardiography, and gynaecologic examination.

We used the HercepTest® (Dako A/S, Glostrup, Denmark) and dual colour fluorescent in situ hybridization (FISH; PathVision® HER2 DNA probe kit, Vysis Inc., Downers Grove, IL, USA) for assessment of Her2 status. Tumours were classed as Her2-positive if they had a staining intensity of +++ on the Herceptest®; if a score of ++ was gained, the tumours were reanalyzed using FISH. Tumours with Her2 gene amplification again were deemed eligible for trastuzumab. Before treatment initiation, an echocardiography was performed; patients with ejection fraction below 50% were excluded from the study. According to our institutional standard, measurement of left ventricular ejection fraction was repeated every six cycles, or when symptoms of congestive heart failure (CHF) occurred.

Treatment plan and patient evaluation

All treatment was administered in an outpatient setting. Gemcitabine was given intravenously at a dose of 1,250 mg/m² on days one and eight; this schedule was repeated in 3-week cycles. Blood count was performed on day one, eight, and fifteen of the first cycle; this was reduced to a single test on day one during consecutive cycles, if no grade III or IV haematological toxicity was observed. If any toxicities ≥ grade II were reported, treatment was delayed for one week or until toxicity resolved to grade I. At the second occurrence, a dose reduction to 75% was performed. In case of grade III adverse events, treatment was held until symptoms resolved to grade I, and gemcitabine dose was immediately reduced to 75% on consecutive cycles.

Trastuzumab was administered at a dose of 8 mg/kg body weight loading dose on the first day of treatment, followed by 6 mg/kg body weight every 3 weeks [33].

Re-evaluation of patients' tumour status was performed with CT-scans of the chest and the abdomen with additional work up if indicated every three cycles according to UICC criteria. Further re-evaluation was performed at any time point when clinical symptoms of disease progression occurred. Complete response (CR) was defined as the disappearance of all measurable lesions for a minimum of 8 weeks. Partial response (PR) was defined as 30% or more reduction in sum of products of the greatest diameters of measurable lesions, no increase of lesion size and no new lesions. Stable disease (SD) was defined as less than 30% decrease and less than 25% increase without the appearance of new lesions. Progressive disease (PD) was defined as greater than 25% increase in tumour size or the appearance of new lesions.

Statistical analysis

Clinical benefit rate (CBR; CR + PR + SD ≥ 6 months) was defined as primary study endpoint; secondary endpoints were time to disease progression (TTP), overall survival (OS), and toxicity.

Based on previous data, a 50% CBR was considered to indicate activity of the combination regimen, and a CBR < 25% was considered unacceptable. The targeted accrual for this study was set at 26 evaluable patients. If ≥11 patients had clinical benefit, a sample size of 26 patients provides statistical power of 80% to reject the null hypothesis that the CBR is <25% with an α of 0.05. CBR and overall response rates are reported with 95% CI. TTP was defined as the interval from the first day of gemcitabine plus trastuzumab application until tumour progression, and OS as the interval from the first day of treatment until death of any cause. Data was analysed as of July 2007. TTP and OS were estimated using the Kaplan–Meier product-limit method. To test the difference between TTP curves in second and beyond second line treatment, the log-rank test was used. A multinomial logistic regression model was utilized to evaluate which factors were potentially associated with a higher probability of achieving CB. As independent factors, we included therapy line (second-line vs. beyond second-line); pre-treatment with anthracyclines, docetaxel, vinorelbine and capecitabine; endocrine receptor status; and CB from the last treatment line before the gemcitabine/trastuzumab regimen. *P* values less than 0.05 were considered to indicate statistical significance. Toxicity was evaluated according to WHO criteria and was recorded per patient as the worst episode that occurred during a cycle of treatment. All statistics were calculated using the statistical package for the social sciences (SPSS®) 12.0 software (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Twenty-nine consecutive patients, median age 60 years (range 29–83 years), diagnosed with metastatic breast cancer were accrued. Table 1 lists the characteristics of the patients. All received gemcitabine and trastuzumab and were included in the intent-to-treat population for safety analysis; as of July 2007, 26 are also evaluable for efficacy

Table 1 Patient characteristics ($n = 29$)

Characteristics	Patients
Entered	29
Karnofsky performance score	80–100
Age (years)	
Median (range)	60 years (range 29–83 years)
Stage (diagnosis)	
I	6 (20.7%)
II	15 (51.7%)
III	4 (13.8%)
IV	3 (10.3%)
Not available	1 (3.4%)
Grading	
1	0
2	10 (34.5%)
3	19 (65.5%)
Ductal/lobular carcinoma	26/3
Oestrogen receptor/progesterone receptor positive	20/9
Her2 Status (IHC/FISH)	28/1
Adjuvant chemotherapy	21 (72.4%)
Adjuvant endocrine therapy	8 (27.6%)
Adjuvant trastuzumab	2 (6.9%)
Palliative endocrine therapy	9 (31%)
Time to recurrence	
Median (range)	26 months (range 7–78 m)
Treatment line (chemotherapy)	
Second line	8 (27.6%)
Third line	8 (27.6%)
Fourth line	10 (34.5%)
Fifth line	3 (10.3%)
Treatment line (trastuzumb)	
Second line	9 (21%)
Third line	9 (31%)
Fourth line	9 (31%)
Fifth line	2 (6.9%)
Prior vinorelbine	28 (96.6%)
Prior vinorelbine + trastuzumab	27 (93.1%)
Prior taxane exposure	21 (72.4%)
Prior taxane + trastuzumab	18 (62.1%)
Prior capecitabine	21 (72.4%)
Prior capecitabine + trastuzumab	20 (69%)
Prior vinorelbine, docetaxel, and capecitabine	13 (44.8%)
Prior trastuzumb (months)	
Median (range)	12 months (6–41 m)
Clinical benefit from the last trastuzumab based treatment line before gemcitabine and trastuzumab	20 (69%)
Patients responding to first-line trastuzumab	18 (62.1%)
Metastatic sites	
Median (range)	3 (range 1–5)
More than one metastatic site	27 (93.1%)
Bones/soft tissue only	6 (20.7%)
Visceral involvement	23 (79.3%)
Brain involvement (at treatment initiation)	5 (17.2%)

IHC Immunohistochemistry, Herceptest® (Dako A/S, Glostrup, Denmark); *FISH* dual colour fluorescent in situ hybridization (PathVision® HER2 DNA probe kit, Vysis Inc., Downers Grove, IL, USA)

analysis. All patients had prior exposure to an anthracycline, trastuzumab, and an anti-microtubule agent (96.6% vinorelbine; 72.4% docetaxel), and 21 pts (72.4%) also to prior capecitabine. Thirteen (44.8%) were pre-treated with all three substances. Of three patients not evaluable for efficacy analysis, one was set off study after receiving one cycle of therapy because it was found that she had received an earlier combination of vinorelbine plus gemcitabine for metastatic disease. Two patients were lost to follow up following two cycles of treatment without any sign of progression or death.

Efficacy

We observed a partial remission in five pts (19.2%, CI [95%] 4.1–34.1%), SD $\geq$ 6 months in 7 (26.9%, CI [95%] 9.9–43.9%), and PD in 14 pts (53.8%, CI [95%] 34.6–73%), translating into a CBR of 46.2% (CI [95%] 27–64.5%) (Table 2).

Median time to progression (TTP) was 3 months (range 1–10, CI [95%] 1.89–4.11) (Fig. 1). Corresponding TTP numbers for second line are 6 months (range 1–7, CI [95%] 4.90–7.10), and 3 months (range 1–10, CI [95%] 2.61–3.39) for beyond second line. Log-rang test revealed no significant difference. Overall survival (OS) was median 17 months (range 4–31 + months, CI [95%] 14.68–19.36).

Four patients (15.4%) developed cerebral metastases as first site of disease progression while on treatment, and five patients with pre-existing cerebral lesions were included. A further three (11.5%) were diagnosed with cerebral metastases during follow up. Of the five patients with pre-existing cerebral disease, only one had a partial remission of brain lesions as treatment response.

In the multivariate analysis (multinomial logistic regression model), none of the factors included (pre-treatment, endocrine receptor status, treatment line, and CB from the last palliative therapy line before study enrolment) were significantly associated with increased probability of achieving CB, although a trend for second line treatment was observed (second vs. beyond second-line; $P = 0.097$).

Table 2 Response rates ($n = 26$)

Number		Response					
		CR	PR	ORR	SD	CBR	PD
Response overall	$n = 26$	–	5 (19.2%)	5 (19.2%)	7 (26.9%)	12 (46.2%)	14 (53.8%)
Response							
2nd line	$n = 8$	–	3 (37.5%)	3 (37.5%)	3 (37.5%)	6 (75%)	2 (25%)
Beyond 2nd line	$n = 18$	–	2 (11.1%)	2 (11.1%)	4 (22.2%)	6 (33.2%)	12 (66.7%)

CR complete response; *PR* partial response; *ORR* overall response rate (CR + PR); *SD* stable disease ≥ 6 months; *CBR* clinical benefit rate (ORR + SD); *PD* progressive disease

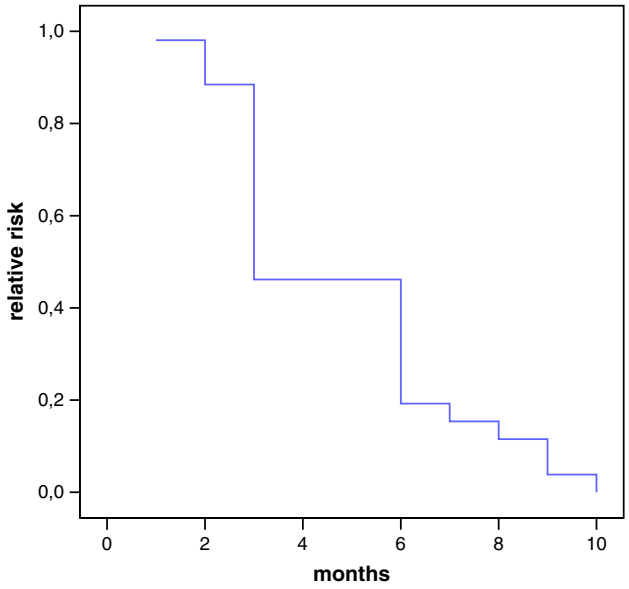

Fig. 1 Time to progression time to progression (TTP) all patients (months)

Toxicity

In general, the combination of gemcitabine plus trastuzumab was reasonably well tolerated and toxicity was manageable. No treatment related deaths were observed. Twenty-nine patients received a total of 145 cycles. Side effects are summarized in Table 3. The main toxicities consisted of neutropenia, thrombocytopenia, and nausea. Notably, no case of CHF or asymptomatic drop in LVEF ≥ 15% occurred in the study population.

Grade 4 neutropenia was observed in two subjects (6.9%), and grade 3 in a further four (13.8%). Thirteen patients experienced grade 1 and 2 neutropenia (44.8%). Grade 3 and 4 thrombocytopenia was found in two patients (6.9%) each, and grade 1 and 2 in a total of six patients (20.7%). No case of grade 4 nausea was reported, however grade 3 nausea was observed in one (3.4%) patient, and a further two suffered from grade 1 and 2 combined (6.9%). No other grade 3 and 4 toxicities were reported. Other side effects (grade 1 and 2 only) included anaemia, asthenia, diarrhoea, fatigue, and headache.

In seven patients (24.1%), a delay of cycles for one week was necessary due to toxicity, mainly neutropenia and thrombocytopenia. Overall, 10/145 (6.9%) cycles had to be delayed for 1 week. In five patients (17.2%), a dose reduction to 75% of initial dosage was necessary.

Discussion

As shown, the combination of gemcitabine and trastuzumab is a feasible and reasonably well-tolerated option in a heavily pre-treated population. Clinical benefit rate and time to progression however were relatively low, and need to be discussed in the light of results from trials of gemcitabine as monotherapy in a similar population, and studies evaluating the role of trastuzumab beyond progression. Furthermore, lapatinib based treatment options need to be taken in account.

When compared to data from gemcitabine in the first- and second-line setting, our results appear disappointing. In one study, an overall response rate of 25% was reported [26]. Similar, in a trial conducted by Bordowicz et al. [27] second-line patients reached an overall response rate of 22%, and a clinical befit rate of 66%. However, when only patients receiving gemcitabine as third-line therapy are taken in account, a lower efficacy becomes evident. In the same study, Brodowicz et al. reported a CBR of 37% in third-line patients. In another trial of gemcitabine after anthracycline and taxane failure, only 26% of patients experienced disease stabilization for a minimum of 4 months, and no case of PR was observed [34]. While cross trial comparisons of phase II studies are usually misleading due to small sample size and heterogeneous patient populations, it appears as if the clinical benefit rate of 46.2% observed in our trial is higher than what would have been expected from gemcitabine monotherapy in a similar setting.

The combination of gemcitabine and trastuzumab itself is not well established, although preclinical data suggested a concentration dependent synergistic effect [35]. In the

Table 3 Toxicities ($n = 29$)

Toxicity	WHO grade			
	I	II	III	IV
Neutropenia	10 (34.5%)	3 (10.3%)	4 (13.8%)	2 (6.9%)
Thrombocytopenia	4 (13.8%)	2 (6.9%)	2 (6.9%)	2 (6.9%)
Anaemia	14 (48.3%)	6 (20.7%)	–	–
Asthenia	–	1 (3.4%)	–	–
Diarrhoea	1 (3.4%)	1 (3.4%)	–	–
Fatigue	8 (27.6%)	4 (13.8%)	–	–
Headache	–	1 (3.4%)	–	–
Muscle pain	6 (20.7%)	–	–	–
Nausea/vomiting	1 (3.4%)	1 (3.4%)	1 (3.4%)	–

largest published clinical trial henceforth, O'Shaughnessy et al. reported a response rate of 38%, with another 36% of enrolled patients experiencing prolonged disease stabilization [36]. Those patients however, while chemotherapy pre-treated, were trastuzumab naïve. Data therefore cannot be directly compared to results from our trial, yet they established in principle the clinical activity of this combination.

As stated above, CBR in our trial is low when compared to trastuzumab containing first-line regimens [13, 37]. Still, results are well in line with data from studies evaluating trastuzumab beyond disease progression [17–18]. TTP, on the other hand, in those studies ranged from 5.2 to 8 months [16–19]. This is considerably longer than the TTP of median 3 months reported here. Still, it needs to be considered that all individuals on our trial had prior exposure to anthracyclines and at least one anti-microtubule agent, and as mentioned, most were pre-treated with capecitabine. Therefore, half of the subjects enrolled on this study were pre-treated with the most effective drugs available in metastatic breast cancer. This is underlined by the fact that response rates and clinical benefit rate was higher in individuals receiving the combination of gemcitabine and trastuzumab as second-line therapy. Also, a trend towards longer TTP in second-line patients was observed.

Toxicity rates observed in our trial compare well to data from different other groups, with neutropenia and thrombocytopenia being the main reasons for treatment delays and dose reduction [26, 34, 36, 38]. Other relevant toxicities consisted of nausea, anaemia and fatigue. In general, treatment was relatively well tolerated. As for the cardiac toxicity of trastuzumab, no case of symptomatic CHF was observed. A drop of left ventricular ejection fraction not necessitating treatment discontinuation was seen in a single patient.

Four patients (15.4%) developed cerebral metastases as first site of progression while on therapy, a problem often encountered in Her2-positive disease [39–41]. This phenomenon is probably caused by the prolongation of survival due to trastuzumab, in connection with the fact that antibodies cannot pass the blood-brain-barrier. A recently published trial of capecitabine with or without lapatinib showed superior results in terms of response rate and time to progression for the combination group. Also, a reduced incidence of brain metastases was observed [15]. As for trastuzumab, there is limited data suggesting a potential benefit of continued trastuzumab treatment following local therapy for brain metastases [42–44]. It is usually suspected that this benefit is rather due to control of systemic disease, and not caused by a direct impact of trastuzumab on cerebral lesions. Also in the study presented here, activity in brain metastases is limited at best, with only one patient deriving clinical benefit from treatment. Therefore, the high incidence of brain metastases in Her2-positive metastatic breast cancer remains a challenging problem.

The above mentioned trial of capecitabine with or without lapatinib that ultimately lead to the FDA approval of this compound, has set a potential new standard for patients with Her2-positive metastatic breast cancer following trastuzumab failure [15]. Yet, most patients enrolled on our study would not have been eligible for that combination, as most (>70%) had already received capecitabine, and only 30% received the combination of gemcitabine and trastuzumab as second line therapy. Therefore, we believe that our data are of value for a specific population of patients, with prior exposure to nearly all of the established treatment options.

We conclude that the combination of gemcitabine and trastuzumab appears to be feasible and safe treatment option. Although clinical benefit rate was relatively low, it needs to be taken in account that this trial accrued heavily pre-treated patients. It appears necessary to evaluate new substances and targets in this specific population in order to further improve outcomes.

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