Gefitinib in Combination with Gemcitabine and Vinorelbine in Patients with Metastatic Breast Cancer Pre-treated with Taxane and Anthracycline Chemotherapy: A Phase I/II Trial

IOANNIS GIOULBASANIS[1], ZACHARENIA SARIDAKI[1], ANTONIA KALYKAKI[1], LAMBROS VAMVAKAS[1], KOSTAS KALBAKIS[1], MICHAIL IGNATIADIS[1], KYRIAKOS AMARANTIDIS[2], STYLIANOS KAKOLYRIS[2], VASSILIS GEORGOULIAS[1] and DIMITRIS MAVROUDIS[1]

[1]*Department of Medical Oncology, University General Hospital of Heraklion;*
[2]*Department of Medical Oncology, University General Hospital of Alexandroupolis, Greece*

Abstract. *Purpose: To determine the tolerability and efficacy of the combination of gefitinib with gemcitabine plus vinorelbine in metastatic breast cancer (MBC) patients, pre-treated with anthracyclines and taxanes. Patients and Methods: Women with measurable MBC pretreated with anthracycline- and taxane-based chemotherapy received oral gefitinib (250 mg/day) continuously combined with intravenous gemcitabine 1000 mg/m^2 and vinorelbine 25 mg/m^2 on day 1, every 2 weeks. The first 10 enrolled patients were evaluated for the safety and tolerability of the proposed fixed-dose regimen. Results: The study was discontinued prematurely due to low accrual. Twenty-five (71%) of the originally scheduled 35 patients received a total of 154 chemotherapy cycles. All the patients had previously received taxane- and 72% additionally anthracycline-based chemotherapy and 64% of them had progressive disease as best response to first-line treatment. Three episodes of dose-limiting toxicities (one non-febrile neutropenia grade 4 and two non-neutropenic infections grade 3) were observed in the safety analysis of the first 10 patients. In an intent-to-treat analysis, the overall response rate was 12% (95% CI, 0-24.7%), the median time to tumour progression was 3.5 months (range 1.0-11.5) and the median overall survival was 10.4 months (range 1.0-46.0). The main toxicity was hematological, with grade 3 and 4 neutropenia occurring in 6 (24%) and 4 (16%) patients, respectively. Febrile neutropenia occurred in 2 (8.0%) patients. Conclusion: Although well tolerated, the gefitinib plus gemcitabine and vinorelbine regimen achieved a low response rate in this prematurely terminated trial and therefore cannot be recommended for women with pre-treated MBC.*

Correspondence to: Dimitris Mavroudis, MD, Ph.D., Department of Medical Oncology, University General Hospital of Heraklion, PO BOX 1352, 71110 Heraklion, Crete, Greece. Tel: +30 2810 392783, Fax: +30 2810 392857, e-mail: georgsec@med.uoc.gr/mavrudis@med.uoc.gr

Key Words: Breast cancer, vinorelbine, gemcitabine, gefitinib, phase I/II study.

Breast cancer is the most common malignancy and the most frequent cause of cancer mortality in women of western countries. Systemic chemotherapy, given as monotherapy or in combinations, is the treatment of choice for women with advanced disease that is hormone receptor-negative or resistant to hormonal therapy and for women with rapidly progressive life-threatening disease. In chemotherapy-naive patients, anthracycline- and/or taxane-based regimens are the preferable treatment options due to their high efficacy, unless contraindicated. However, there is an increasing number of patients who have already been treated with these agents in the adjuvant or in the first-line metastatic setting and therefore new therapeutic regimens are warranted for the subsequent treatment of these patients.

No standard effective treatment exists after failure of both anthracycline- and taxane-based therapy in patients with metastatic breast cancer (MBC) and the therapeutic options are limited (1). Moreover, no randomized trial has ever demonstrated an overall survival benefit after second-line chemotherapy (2). Newer cytotoxic agents, such as gemcitabine, capecitabine and vinorelbine, are among the available choices (3-5). Combination chemotherapy, compared with sequential single agent therapy, although more toxic, has generally no significant difference in activity or efficacy (6-10). The combination of gemcitabine plus vinorelbine is attractive due to the activity of each individual agent and the lack of overlapping toxicities. Based on these theoretical advantages, the combination has been evaluated in MBC patients as salvage therapy, in several phase II trials (11-18). In most of these studies, the cycles were repeated every 21 days, while in one study (14) both drugs were administered every 15 days. In the latter study, the efficacy

was comparable to the other trials, but the observed toxicity profile was relatively more favourable. Furthermore, in a recent phase III study, the combination of the two drugs resulted in better progression-free survival compared with vinorelbine alone (19).

Breast carcinomas express the epidermal growth factor receptor (EGFR/HER1) in 14-91% of cases and these tumors exhibit a more aggressive biological behaviour, by means of increased capacity for invasion and metastasis (20). As a consequence, EGFR-expressing breast carcinomas are associated with a worse clinical outcome (21), although standardization of EGFR testing is problematic and the prognostic value of EGFR expression in breast cancer remains controversial (22-24). Gefitinib (ZD1839, Iressa®), a reversible EGFR tyrosine kinase inhibitor (25), has a wide spectrum of antitumor activity including in breast cancer (26). Preclinical studies have suggested that the combination of gefitinib with different cytotoxic drugs may enhance their effectiveness (26, 27).

On the basis of these data, a multicenter, open-label, non-comparative, two-step phase I/II trial was conducted, in order to evaluate the tolerability and activity of the combination of gefitinib plus gemcitabine and vinorelbine in women with MBC pre-treated with anthracycline- and taxane-based chemotherapy.

Patients and Methods

Eligibility. Female patients aged 18 to 75 years, with histologically-confirmed MBC, previously treated with taxane- and/or anthracycline-based chemotherapy for metastatic disease or as adjuvant/neoadjuvant treatment, with disease relapse or progression within six months from completion of chemotherapy, were eligible for the study. All the patients were required to have measurable, non-irradiated disease according to Response Evaluation Criteria in Solid Tumors (RECIST) (28), a World Health Organisation (WHO) performance status of 0 to 2, predicted life expectancy ≥12 weeks and good hematological, hepatic and renal function. Patients with brain metastases were allowed to participate if they had received cranial irradiation with radiological and clinical improvement or stabilization. No other concurrent investigational treatment was allowed. All the patients had to sign a written informed consent before study entry. The study was approved by the Ethics and Scientific Committees of our Institution.

Pre-treatment evaluation and follow-up. Patient evaluation included a detailed medical history and physical examination, a complete blood cell count with differential and platelet counts, whole blood chemistry, urinalysis and electrocardiography. All sites of measurable disease were initially documented by computed tomography scan or magnetic resonance imaging within a period of three weeks before study entry. Other imaging modalities were performed according to symptoms and the physician's clinical judgement.

During the study treatment, patient monitoring included the assessment of concurrent illness and adverse events at all visits. Complete blood cell count was performed weekly while whole blood chemistry and physical examination were performed before each chemotherapy administration. Tumor assessment was performed by repeating computed tomography scans or magnetic resonance imaging studies at the end of cycles 4, 8 and 12.

Responders, who continued treatment with the biological agent alone at the end of 12 chemotherapy cycles, were followed with physical examination, hematology and biochemistry tests, adverse events monitoring and tumor assessment studies every 12 weeks until disease progression.

Treatment plan. Vinorelbine (Navelbine®; Pierre Fabre, Paris, France) 25 mg/m^2, diluted in 50 ml of normal saline was administered over a 10 min intravenous infusion on day 1 of each cycle followed by gemcitabine (Gemzar®; Eli Lilly, Indianapolis, USA) 1000 mg/m^2 diluted in 250 ml of normal saline as an intravenous infusion over 30 min. Routine antiemetic prophylaxis with a 5-hydroxytryptamine-3-receptor antagonist was used, 30 min prior to chemotherapy. Gefitinib (ZD1839, Iressa®; AstraZeneca, London, United Kingdom) 250 mg as film-coated tablets was administered orally continuously, once daily. A total of 12 treatment cycles were administered and each chemotherapy cycle consisted of 14 days. Responding patients and those who had their disease stabilized at the end of chemotherapy cycles could continue to receive gefitinib monotherapy, at the same dose of 250 mg, until disease progression, unacceptable toxicity or withdrawal of consent. Treatment was routinely given on an out-patient basis.

Dose delays. Chemotherapy administration was interrupted for up to one week in the case of incomplete recovery from the toxicity of the previous chemotherapy cycle on the scheduled day of treatment. In the event of skin toxicity grade ≥2 according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (29) or diarrhea grade 3, gefitinib was discontinued for up to a maximum of 14 days until resolution of toxicities or until toxicities decreased in severity to grade 1. In the case of diarrhea grade ≥2 accompanied by neutropenia grade 3 or 4, gefitinib was interrupted for up to a maximum of 14 days, until the absolute neutrophil count (ANC) was $>0.5\times10^9$/l with resolution of the diarrhea, or the diarrhea had decreased in severity to grade 1.

Dose reductions. The doses of gemcitabine and vinorelbine were reduced by 25% if patients developed neutropenia or thrombocytopenia grade 4, lasting for more than 5 days, non-hematological toxicity grade 3 or 4 (other than grade 3 nausea/ vomiting that could be prevented by premedication) or febrile neutropenia (oral temperature >38˚C with ANC $<1.0\times10^9$/l). Additionally, if chemotherapy treatment was postponed twice, either consecutively or in subsequent chemotherapy cycles, because of delayed recovery from previous toxicity attributed to these drugs, the doses of vinorelbine and gemcitabine were also reduced by 25%. No dose reduction of gefitinib was allowed.

Discontinuation of treatment. If, despite dose reduction, the patient developed grade 4 hematological or grade 3 or 4 non-hematological toxicity or had not fully recovered from the toxicity of the previous chemotherapy administration on the scheduled day of treatment, the treatment was discontinued permanently.

Dose-limiting toxicities. Dose-limiting toxicity (DLT) was defined as any of the following toxicities occurring during the first month of treatment (*i.e.* the first 2 cycles): grade 4 neutropenia or

thrombocytopenia lasting for more than 5 days; non-hematological toxicity grade 3 or 4 (other than grade 3 nausea/vomiting or grade 3 rash); febrile neutropenia (oral temperature >38˚C with ANC $<1.0\times10^9$/l); significant corneal epithelial change (≥ grade 2) and any treatment delay for more than one week due to unresolved toxicity.

Study end-points. The primary objective of the trial was the evaluation of activity of the gemcitabine plus vinorelbine with oral gefitinib combination, in women with MBC pre-treated with taxane and anthracycline chemotherapy by estimating the objective response rate. Secondary objectives were the estimation of: disease control rate (complete response, partial response and stable disease), progression-free and overall survival and the evaluation of acute (including DLT) and cumulative toxicity, as well as overall tolerability of the combination.

Tumor response was defined according to the standardized response definitions of the RECIST criteria (28). CT scans of the target lesion(s) were performed at baseline and every 4 cycles of chemotherapy (every 8 weeks) and every three months after the end of treatment. Tumor responses were confirmed by a second evaluation at least four weeks later. Toxicity was evaluated and graded according to NCI-CTC criteria (29).

Statistical design and analysis. This was an open-label, non-comparative, multicenter, two-step phase I/II trial. Recruitment proceeded in two steps to ensure the safety of the combination. Initially, 10 patients were enrolled to evaluate the safety and tolerability of the proposed fixed-dose regimen of combination therapy. A full safety evaluation was conducted for the initial 10 patients after 2 cycles (4 weeks) of combination therapy before further patients were treated. If 3 or fewer patients experienced DLT during the first month of treatment, then the study would proceed to the phase II part and the patient cohort would be expanded to 35 patients for evaluation of the efficacy of the combination. If four or more patients of the initial 10 patients experienced DLT, the dose of gemcitabine and vinorelbine would be reduced by 25% and a further 10 patients would be recruited at the new dose level. If tolerated well, this lower dose level would be expanded to 35 patients. If the combination at the lower dose was not well tolerated, the trial would be terminated.

The Fleming method was used to calculate the number of patients required (30). A sample size of 35 patients was sufficient to give an 80% probability of rejecting a baseline response rate of 20% with an exact 5% one-sided significance test when the true response was at the clinically relevant rate of 40%. The hypothesis that the response rate was equal to or less than the baseline would be rejected if 12 or more responses were observed out of the 35 patients. The safety analysis and the intention-to-treat population consisted of all the patients who were enrolled in the study. The analysis population for all efficacy end-points was the intention-to-treat population. The standard summary statistics for discrete variables were: count and proportion. The response rates and controlled disease rates were summarised by proportions together with a 95% confidence interval (CI) and the objective response rate would also have a 90% CI calculated. Durations of progression-free survival and overall survival were estimated by Kaplan-Meier methods. The trial treatment would be deemed to have clinically relevant activity if the hypothesis that the objective response rate was 20% was rejected in favor of a higher objective response rate.

Table I. *Patient characteristics*.

	N=25	%
Median age (years) (min – max)	55 (27-75)	
Performance status		
0	8	32
1	16	64
2	1	4
ER / PR / HER2 status		
ER/PR- positive, HER2 positive	2	8
ER/PR-positive, HER2 negative	10	40
ER/PR-negative, HER2-positive	4	16
ER/PR-negative, HER2-negative	4	16
Unknown status	5	20
Prior therapy		
Surgery	23	92
Radiotherapy	11	44
Adjuvant hormonotherapy	19	76
Adjuvant chemotherapy	18	72
Line of therapy		
2nd	8	32
3rd	9	36
≥4th	8	32
Response to 1st-line treatment		
PR	2	8
SD	7	28
PD	16	64
No. of organs involved		
1	14	56
2	8	32
≥3	3	12
Organs involved		
Bones	6	24
Nodes	5	4
Lung	9	36
Liver	15	60
Skin	6	24
Central nervous system	2	8

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal receptor 2. PR: partial response, SD: stable disease, PD: progressive disease.

Results

Patient demographics. Between August 2002 and January 2006, twenty-five (71%) of the originally scheduled 35 patients with MBC were recruited in two centers. The study was then closed prematurely due to low accrual. The patients' demographic characteristics are given in Table I. All the patients had received taxanes and most of them (72%) had also received anthracyclines in the adjuvant/neoadjuvant or in the first-line setting. Almost all the patients (92%) had visceral disease. Two (8%) patients had metastasis in the central nervous system. Notably, the majority of patients (64%) had progressive disease as best response to first-line treatment.

Table II. *Adverse events related to study treatment.*

	Grade I		Grade II		Grade III		Grade IV	
	N	%	N	%	N	%	N	%
Neutropenia	2	8	5	20	6	24	4	16
Febrile neutropenia	-	-	-	-	-	-	2	8
Anemia	12	48	6	24	1	4	-	-
Thrombocytopenia	6	24	1	4	-	-	-	-
Infection without neutropenia	-	-	1	4	2	8	-	-
Fever without infection	2	8	1	4	-	-	-	-
Nausea	7	28	2	8	-	-	-	-
Vomiting	1	4	-	-	-	-	-	-
Diarrhea	6	24	1	4	1	4	-	-
Stomatitis	3	12	-	-	-	-	-	-
Allergy	2	8	1	4	1	4	-	-
Constipation	3	12	-	-	-	-	-	-
Neurotoxicity	3	12	-	-	-	-	-	-
Skin toxicity	7	28	1	4	1	4	-	-
Asthenia	3	12	1	4	1	4	-	-
Conjuctivitis	2	8	-	-	-	-	-	-
Liver toxicity	2	8	-	-	-	-	-	-

N=number of patients.

Treatment administration. All the patients received at least one administration of chemotherapy and were assessable for toxicity. A total of 154 chemotherapy cycles were administered (median 5 cycles per patient, range 1-12). Twenty-six (17%) cycles were delayed due to hematological (n=14), non-hematological (n=3) toxicity or both (n=1) and late admission (n=8). In 6 (24%) cycles, the dose was decreased due to hematological toxicity. Two (8%) patients completed their treatment as per protocol. The main reason for treatment discontinuation was disease progression (60%). There was one major protocol violation (consent withdrawal) and this patient was included in the intention-to-treat analysis. No treatment-related death occurred during the study and all the deaths were due to disease progression.

Toxicity. In the initial part of the study, assigned for the evaluation of safety and tolerability of the regimen, non-febrile neutropenia grade 4 occurred in one patient while two other patients developed non-neutropenic, febrile infection grade 3; all the patients required hospitalisation for intravenous administration of antibiotic therapy and all recovered uneventfully. The rest of the recorded toxicities were mild (grade ≤2). Since no more than three patients experienced DLT, the trial expanded and the rest of the patients entered the study as scheduled.

All grades of treatment-related toxicity for the enrolled 25 patients are listed in Table II. Neutropenia was the main toxicity, with an incidence of grade 3 and 4 in six (24%) and four (16%) patients, respectively. Febrile neutropenia occurred in two (8.0%) patients. The other hematological toxicities were mild (Table II). One (4%) patient developed a grade 3 acne-like rash, that resolved one week after gefitinib treatment suspension, but subsequently experienced disease progression and the treatment was discontinued. Only one (4%) patient discontinued therapy due to a treatment-related adverse event (febrile neutropenia grade 4).

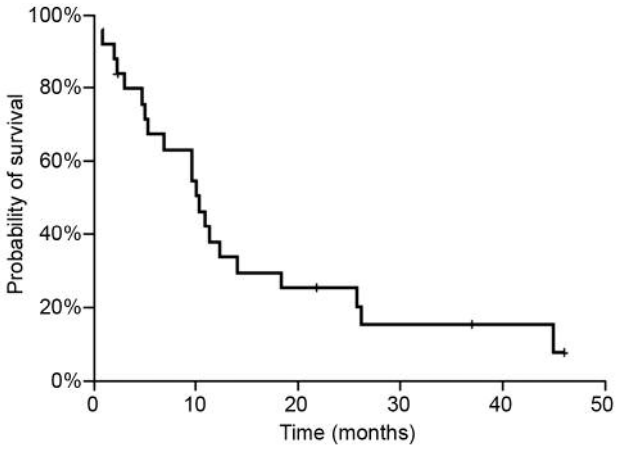

Figure 1. *Kaplan-Meier probability of survival for the 25 patients treated with the vinorelbine-gemcitabine and gefitinib combination.*

Clinical activity. The median follow-up period of the study was 10.1 months (range 1.0-46.0). On an intention-to-treat analysis of the 25 patients enrolled in the trial, no complete response was recorded while three (12%) patients achieved a confirmed objective partial response, resulting in an overall response rate of 12% (95% CI, 0-24.7%). Seven (28%) patients had stable disease, while 15 (60%) patients experienced disease progression. The duration of response for the three responding patients was 3.1, 5.9 and 8.4 months. The median time to tumor progression was 3.5 months (range 1.0-11.5). The median overall survival was 10.1 months (range 1.0-46.0) and the estimated 1-year survival rate was 37.8% (Figure 1).

Discussion

To the best of our knowledge, this is the first study evaluating the tolerability and efficacy of the combination of gemcitabine plus vinorelbine and gefitinib, an oral EGFR tyrosine kinase inhibitor, in women with MBC previously treated with anthracyclines and taxanes. The chemotherapy combination of gemcitabine plus vinorelbine has been previously explored in phase I/II studies and in various treatment plans (11-18). The regimen showed promising anti-tumor activity with response rates ranging from 22% to 58%, depending on previous exposure to chemotherapy. The major dose-limiting toxicity was myelosuppression.

In some recent phase II trials (17, 18, 31), the efficacy of the gemcitabine and vinorelbine combination was evaluated in patients previously treated with anthracyclines alone and/or taxanes, with response rates ranging from 33 to 44%. Activity as well as toxicity was similar in the subgroups of patients pretreated with either combinations of taxanes and anthracyclines or anthracyclines alone, at least in one trial (17). Moreover, in a phase III trial, 252 women with advanced breast cancer, pretreated with anthracyclines and taxanes, were randomized to receive vinorelbine alone or in combination with gemcitabine (19). Patients assigned to the combination arm had better progression-free survival; however, this did not translate into a significant difference in overall survival. In that study, vinorelbine was administered at 30 mg/m^2 and gemcitabine at 1200 mg/m^2, on days 1 and 8 in cycles every 21 days.

In pre-clinical studies and animal models, gefitinib could reverse resistance through inhibition of the breast cancer resistance protein, at least when combined with some chemotherapeutic drugs, such as SN-38 and topotecan (32). Nevertheless, others have shown conflicting results for the combination of gefitinib with cytotoxic agents such as cisplatin, gemcitabine, oxaliplatin, treosulfan and treosulfan plus gemcitabine (33). The *in vitro* models suggested that gefitinib may have differential effects in response to concomitant cytotoxic chemotherapy. The mechanism involved may relate to the effect of tyrosine kinase inhibitors (TKIs) on growth rate *versus* their effect on the ability of the cell to survive the stimulus to apoptosis produced by chemotherapy (33).

Despite the initial promising results of an early open-label study in patients with MBC (34), gefitinib monotherapy at 500 mg/day was not efficacious in women with breast cancer pre-treated with taxane- and anthracycline-based combinations (35). The same observation in a similar patient population was extended to erlotinib, another EGFR tyrosine kinase inhibitor (36). However, there appears to be subgroup of tamoxifen-resistant, estrogen receptor-positive women who might gain some benefit from gefitinib treatment (20).

The combination of vinorelbine and gefitinib in squamous cell carcinoma of head and neck cell lines resulted in a supra-additive or additive cytotoxic effect demonstrating the possible benefit of combining an EGFR-targeting compound with a cell cycle specific drug (37). Despite the disappointing results in patients with lung cancer, for the combination of gefitinib with either gemcitabine and cisplatin (38) or paclitaxel and carboplatin (39), some other encouraging data of gefitinib treatment alone or with low dose vinorelbine given every 2 weeks have been reported in Chinese patients with adenocarcinoma of the lung who had failed at least 2 prior chemotherapy regimens (40). The co-administration of gefitinib either with single agents (epirubicin, docetaxel) or with combinations (paclitaxel-carboplatin, epirubicin-paclitaxel) in unselected breast cancer patients was tested in some phase I/II studies and generally did not seem to add any substantial benefit (41-44).

Although the present study was closed prematurely due to low accrual, the observed response rate (12%) in the intent-to-treat population did not satisfy the initial statistical hypothesis, so the combination of gefitinib with vinorelbine and gemcitabine was deemed to be ineffective in patients with MBC pre-treated with anthracyclines and taxanes. The observed toxicities related to the tyrosine kinase inhibitor were as expected. However, the bi-weekly administration of the cytotoxic drugs was less toxic compared with the usual combination of these agents given in a 21-day cycle. This is in accordance with another, similarly designed, phase II trial where the observed response rate was higher (54%), probably due to the fact that only 50% of these patients had been previously exposed to taxanes (14). Moreover, in our study, the incidence of febrile neutropenia was not a major limitation of the combination, unlike one previous trial where gefitinib seemed to exacerbate the neutropenic effect of vinorelbine (45). Furthermore, in the Chinese study cited above, the addition of gefitinib to low-dose vinorelbine was not associated with a high incidence of febrile neutropenia in pre-treated patients with lung cancer (40).

Although most of the patients' primary tumors were tested for ER/PR and HER2 expression, a major limitation of our study was that the EGFR status and the associated downstream molecular pathway were not evaluated. There is some evidence that basal-like breast carcinomas overexpress EGFR and theoretically these patients might gain some benefit from EGFR blocking agents (46). In the present study, only one out of four patients with basal-like (triple negative) breast cancer experienced disease stabilization as best response and the other three progressed while on treatment. Furthermore, even the higher dose (500 mg) of gefitinib monotherapy was ineffective in a similar cohort of taxane- and anthracycline-pretreated breast cancer patients and there was no correlation between EGFR expression and response to gefitinib (35). The mitogen-activated protein kinase (MAPK) pathway has been implicated for resistance to gefitinib at least in breast cancer cell lines and thus might explain, at least in part, the above results (47). Of note, Feng *et al*. have recently demonstrated that gemcitabine causes EGFR degradation and this may be one of its mechanisms of action, thus rendering the role of gefitinib in this particular combination even more questionable (48).

The most solid data concerning the efficacy of the combination of vinorelbine and gemcitabine in women pre-treated with anthracyclines and taxanes emerged from a recently published phase III trial (19) in which higher doses of both chemotherapeutic agents were used. The observed response rate was 36% (95% CI: 28-45%), progression-free survival was 6.0 months (95% CI: 4.8-

7.1) and overall survival was 15.9 (95% CI: 12.6-19.1) months. In comparison with these data, the results of the present study indicated that the addition of gefitinib to the vinorelbine-gemcitabine regimen yielded inferior responses and survival. Some possible explanations for these differences could be the small sample size of the present trial, the different design and doses of chemotherapy administration, the aggressive biological behavior of the tumor in the majority of enrolled patients (as 64% of them experienced disease progression in the first-line setting and most of them had visceral involvement), a possible antagonistic effect between the tyrosine kinase inhibitor and the cytotoxic combination and, last but not least, the inability to select those patients who would be more likely to gain a significant clinical benefit from this combined treatment. However, based on our results, we do not recommend the use of this regimen in women with pre-treated MBC.

Acknowledgements

This study was supported by AstraZeneca (trial code 1839IL/0112).

References

1 Colozza M, de Azambuja E, Personeni N, Lebrun F, Piccart MJ and Cardoso F: Achievements in systemic therapies in the pregenomic era in metastatic breast cancer. Oncologist *12*: 253-270, 2007.

2 Cardoso F, Di LA, Lohrisch C, Bernard C, Ferreira F and Piccart MJ: Second and subsequent lines of chemotherapy for metastatic breast cancer: What did we learn in the last two decades? Ann Oncol *13*: 197-207, 2002.

3 Ershler WB: Capecitabine monotherapy: safe and effective treatment for metastatic breast cancer. Oncologist *11*: 325-335, 2006.

4 Aapro MS, Harper P, Johnson SA and Vermorken JB: Developments in cytotoxic chemotherapy: advances in treatment utilising vinorelbine. Crit Rev Oncol Hematol *40*: 251-263, 2001.

5 Heinemann V: Gemcitabine in metastatic breast cancer. Expert Rev Anticancer Ther *5*: 429-443, 2005.

6 Chlebowski RT, Smalley RV, Weiner JM, Irwin LE, Bartolucci AA and Bateman JR: Combination *versus* sequential single agent chemotherapy in advanced breast cancer: associations with metastatic sites and long-term survival. The Western Study Group and The Southeastern Cancer Study Group. Br J Cancer *59*: 227-230, 1989.

7 Joensuu H, Holli K, Heikkinen M, Suonio E, Aro AR, Hietanen P *et al*: Combination chemotherapy single-agent therapy as first- and second-line treatment in metastatic breast cancer: a prospective randomized trial. J Clin Oncol *16*: 3720-3730, 1998.

8 Koroleva I, Wojtukiewicz M, Zaluski J *et al*: Preliminary results of a phase II randomized trial of taxotere (T) and doxorubicin (A) given in combination or sequentially as first-line chemotherapy (CT) for metastatic breast cancer (MBC). Proc Am Soc Clin Oncol *20*: 30a (abstr 117) 2001.

9 Alba E, Martin M, Ramos M, Adrover E, Balil A, Jara C *et al*: Multicenter randomized trial comparing sequential with concomitant administration of doxorubicin and docetaxel as first-line treatment of metastatic breast cancer: a Spanish Breast Cancer Research Group (GEICAM-9903) phase III study. J Clin Oncol *22*: 2587-2593, 2004.

10 Conte PF, Guarneri V, Bruzzi P, Prochilo T, Salvadori B, Bolognesi A *et al*: Concomitant *versus* sequential administration of epirubicin and paclitaxel as first-line therapy in metastatic breast carcinoma: results for the Gruppo Oncologico Nord Ovest randomized trial. Cancer *101*: 704-712, 2004.

11 Haider K, Kornek GV, Kwasny W, Weinländer G, Valencak J, Lang F *et al*: Treatment of advanced breast cancer with gemcitabine and vinorelbine plus human granulocyte colony-stimulating factor. Breast Cancer Res Treat *55*: 203-211, 1999.

12 Nicolaides C, Dimopoulos MA, Samantas E, Bafaloukos D, Kalofonos C, Fountzilas G *et al*: Gemcitabine and vinorelbine as second-line treatment in patients with metastatic breast cancer progressing after first-line taxane-based chemotherapy: phase II study conducted by the Hellenic cooperative Oncology Group. Ann Oncol *11*: 873-875, 2000.

13 Valenza R, Leonardi V, Gebbia V and Agostara B: Gemcitabine and vinorelbine in pretreated advanced breast cancer: a pilot study. Ann Oncol *11*: 495-496, 2000.

14 Stathopoulos GP, Rigatos SK, Pergantas N, Tsavdarides D, Athanasiadis I, Malamos NA *et al*: Phase II trial of biweekly administration of vinorelbine and gemcitabine in retreated advanced breast cancer. J Clin Oncol *20*: 37-41, 2002.

15 Moser R, Hausmaninger H, Mlineritsch B, Steger G, Haidinger R, Thaler J *et al*: Gemcitabine and vinorelbine as first or second line therapy in patients with advanced breast cancer: a prospective phase II trial. Proc Am Soc Clin Oncol *20*: 56b (abstr 1973) 2001.

16 Sanal SM, Gokmen E, Karabulut B and Sezgin C: Gemcitabine and vinorelbine combination in patients with metastatic breast cancer. Breast J *8*: 171-176, 2002.

17 Morabito A, Filippelli G, Palmeri S, Cascinu S, Ferraù F, Zagonel V *et al*: The combination of gemcitabine and vinorelbine is an active regimen as second-line therapy in patients with metastatic breast cancer pre-treated with taxanes and/or anthracyclines: a phase I-II study. Breast Cancer Res Treat *78*: 29-36, 2003.

18 Donadio M, Ardine M, Berruti A, Ritorto G, Fea E, Mistrangelo M *et al*: Gemcitabine and vinorelbine as second-line treatment in patients with metastatic breast cancer: a phase II study. Cancer Chemother Pharmacol *52*: 147-152, 2003.

19 Martin M, Ruiz A, Munoz M, Balil A, García-Mata J, Calvo L *et al*: Gemcitabine plus vinorelbine *versus* vinorelbine monotherapy in patients with metastatic breast cancer previously treated with anthracyclines and taxanes: final results of the phase III Spanish Breast Cancer Research Group (GEICAM) trial. Lancet Oncol *8*: 219-225, 2007.

20 Agrawal A, Gutteridge E, Gee JM, Nicholson RI and Robertson JF: Overview of tyrosine kinase inhibitors in clinical breast cancer Endocr Relat Cancer *12*: 135-144, 2005.

21 Klijn JG, Berns PM, Schmitz PI and Foekens JA: The clinical significance of epidermal growth factor receptor in human breast cancer: a review of 5232 patients. Endocr Rev *13*: 3-17, 1992.

22 Tsutsui S, Ohno S, Murakami S, Hachitanda Y and Oda S: Prognostic value of epidermal growth factor and its relationship to the ER status of 1029 patients with breast cancer. Breast Cancer Res and Treat *71*: 67-75, 2002.
23 Ferrero JM, Ramaioli A, Largillier R, Formento JL, Francoual M, Ettore F *et al*: Epidermal growth factor receptor expression in 780 breast cancer patients: a reappraisal of the prognostic value based on an eight-year median follow-up. Ann Oncol *12*: 841-846, 2001.
24 Rampaul RS, Pinder SE, Wencyk PM, Nicholson RI, Blamey RW, Robertson JF *et al*: EGFR expression in operable breast cancer: is it of prognostic significance? Clin Cancer Res *10*: 2578, 2004.
25 Herbst RS, Fukuoka M and Baselga J: Gefitinib, a novel targeted approach to treating cancer. Nat Rev Cancer *4*: 956-965, 2004.
26 Ciardiello F, Caputo R, Bianco R, Damiano V, Pomatico G, De Placido S *et al*: Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa®), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. Clin Cancer Res *6*: 2053-2063, 2000.
27 Sirotnak FM, Zakowski MF, Miller VA, Scher HI and Kris MG: Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by co-administration of ZD1839 (Iressa®), an inhibitor of EGFR tyrosine kinase. Clin Cancer Res *6*: 4885-4892, 2000.
28 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L *et al*: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92: 205-216, 2000.
29 National Cancer Institute Common Toxicity Criteria, Version 2.0, 1999.
30 Fleming TR: One-sample multiple testing procedure for phase II clinical trials. Biometrics *38*: 143-151, 1982.
31 Lobo F, Virizuela JA, Dorta FJ, Florián J, Lomas M, Jiménez E *et al*: Gemcitabine/vinorelbine in metastatic breast cancer patients previously treated with anthracyclines: results of a phase II trial. Clin Breast Cancer *4*: 46-50, 2003.
32 Yanase K, Tsukahara S, Asada S, Ishikawa E, Imai Y and Sugimoto Y: Gefitinib reverses breast cancer resistance protein-mediated drug resistance. Mol Cancer Ther *23*: 1119-1125, 2004.
33 Knight LA, Di Nicolantonio F, Whitehouse P, Mercer S, Sharma S, Glaysher S *et al*: The *in vitro* effect of efitinib ('Iressa') alone and in combination with cytotoxic chemotherapy on human solid tumours. BMC Cancer *4*: 83, 2004.
34 Albain K, Elledge R, Gradishar W, Hayes DF, Rowinsky E, Hudis C *et al*: Open-label, phase II, multicenter trial of ZD1839 ('Iressa') in patients with advanced breast cancer. Breast Cancer Res and Treat *76(suppl 1)*: S33 (abstr 20) 2002.
35 von Minckwitz G and Jonat W: A multicentre phase II study on gefitinib in taxane- and anthracycline – pretreated metastatic breast cancer. Breast Cancer Res and Treat *89*: 165-172, 2005.
36 Winer E, Cobleigh M, Dickler M, Miller K, Fehrenbacher L, Jones C *et al*: Phase II multicenter study to evaluate the efficacy and safety of Tarceva (erlotinib, OSI-774) in women with previously treated locally advanced or metastatic breast cancer. Breast Cancer Res and Treat *76(suppl 1)*: S115, (abstr 445) 2002.
37 Erjala K, Raitanen M, Kulmala J and Grénman R: Concurrent use of vinorelbine and gefitinib induces supra-additive effect in head and neck squamous cell carcinoma cell lines. J Cancer Res Clin Oncol *133*: 169-176, 2007.
38 Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V *et al*: Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial-INTACT 1. J Clin Oncol *22*: 777-784, 2004.
39 Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C *et al*: Gefitinib in combination with paclitaxel and carboplatin in advanced non-small cell lung cancer: a phase III trial-INTACT 2. J Clin Oncol *22*: 785-794, 2004.
40 Chen YM, Liu JM, Chou TY, Perng RP, Tsai CM, Whang-Peng J *et al*: Phase II randomized study of daily gefitinib treatment alone or with vinorelbine every 2 weeks in patients with adenocarcinoma of the lung who failed at least 2 regimens of chemotherapy. Cancer *109*: 1821-1828, 2007.
41 Gasparini G, Sarmiento R, Amici S, Longo R, Gattuso D, Zancan M *et al*: Gefitinib (ZD1839) combined with weekly epirubicin in patients with metastatic breast cancer: a phase I study with biological correlate. Ann Oncol *6*: 1867-1873, 2005.
42 Ciardiello F, Troiani T, Caputo F, De Laurentiis M, Tortora G, Palmieri G *et al*: Phase II study of gefitinib in combination with docetaxel as first-line therapy in metastatic breast cancer. Br J Cancer *94*: 1604-1609, 2006.
43 Fountzilas G, Pectasides D, Kalogera-Fountzila A, Skarlos D, Kalofonos HP, Papadimitriou C *et al*: Paclitaxel and carboplatin as first-line chemotherapy combined with gefitinib (IRESSA®) in patients with advanced breast cancer: a phase I/II study conducted by the Hellenic Cooperative Oncology Group. Breast Cancer Res Treat *92*: 1-9, 2005.
44 Guarneri V, Frassoldati A, Ficarra G, Puglisi F, Andreetta C, Michelotti A *et al*: Phase II, randomized trial of preoperative epirubicin-paclitaxel +/– gefitinib with biomarker evaluation in operable breast cancer. Breast Cancer Res Treat *110*: 127-134, 2008.
45 Pujol JL, Viens P, Rebattu P, Laurie SA, Feld R, Deneulin A *et al*: Gefitinib (IRESSA®) with vinorelbine or vinorelbine/cisplatin for chemotherapy-naive non-small cell lung cancer patients. J Thorac Oncol *1*: 417-424, 2006.
46 Kinkor Z: Basal-like carcinoma of the breast – the actual review and clinico-pathological correlations. Ceska Gynekol *72*: 351-354, 2007.
47 Normanno N, De Luca A, Maiello MR, Campiglio M, Napolitano M, Mancino M *et al*: The MEK/MAPK pathway is involved in the resistance of breast cancer cells to the EGFR tyrosine kinase inhibitor gefitinib. J Cell Physiol *207*: 420-427, 2006.
48 Feng FY, Varambally S, Tomlins SA, Chun PY, Lopez CA, Li X *et al*: Role of epidermal growth factor receptor degradation in gemcitabine-mediated cytotoxicity. Oncogene *26*: 3431-3439, 2007.

Received April 15, 2008
Revised July 18, 2008
Accepted August 8, 2008