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Phase **I-II trial of prolonged gemcitabine infusion plus paclitaxel as a biweekly schedule for advanced breast cancer patients pretreated with anthracyclines**

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ABSTRACT

Purpose Paclitaxel (PACL) plus gemcitabine (GEM) is an effective regimen for advanced breast cancer patients pretreated with anthracyclines. A prolonged GEM infusion at a fixed-dose rate (FDR) of 10 mg/m²/min produces higher levels of intracellular active metabolites of GEM compared with a standard 30-minute infusion. In the present phase I/II trial we investigated the association of FDR GEM plus PACL. **Methods** 1,200 mg/m² was the dose of GEM recommended for the phase II study, in which patients received PACL at 150 mg/m², followed by FDR GEM at 1,200 mg/m² (total GEM infusion time = 120 minutes), both drugs administered biweekly. **Results** Forty-two anthracycline-pretreated advanced breast cancer patients with disease recurrence following at least one line of chemotherapy were enrolled. Two (4.8%) and 12 (33.3%) patients experienced a complete and partial response, respectively, for an overall response rate of 38.1% (95% CI, 23.4%-52.8%). Median progression free survival and overall survival were 5 and 19.9 months, respectively. No statistically significant association was noted between *in situ* protein expression of RRM1 and BRCA1 (as assessed by immunofluorescence combined with automated quantitative analysis) and response to treatment in 15 patients with tissue available for analysis. Toxicity was mostly mild to moderate, mainly consisting of G3-G4 neutropenia (9.6%) and hypertransaminasemia (9.5%). **Conclusions** Biweekly FDR GEM in combination with PACL is an active and safe regimen for advanced breast cancer patients pretreated with anthracyclines. A prolonged infusion regimen of GEM does not seem to improve the efficacy of a standard 30-minute infusion.

Key words: advanced breast cancer; fixed-dose rate; gemcitabine; paclitaxel; HER2-negative breast cancer.

INTRODUCTION

Over the last 15 years, the widespread use of anthracyclines in the early stages of breast cancer has changed the clinical scenario of the advanced setting, with many patients developing an anthracycline-refractory disease. Therefore, innovative and active anthracycline-free combinations are needed. Taxanes are among the most active drugs in breast cancer and are widely employed both in the early and advanced setting, either as single-agents or in combination regimens [1]. During the past two decades, many other effective cytotoxics have been introduced in the clinic, including vinorelbine, capecitabine and gemcitabine, thus increasing the therapeutic armamentarium for the management of advanced breast cancer. Among them, gemcitabine (GEM), a deoxycytidine-analogue antimetabolite, undergoes intracellular phosphorylation into GEM diphosphate and GEM triphosphate, the two active metabolites to whom the cytotoxic properties of GEM are attributed [2]. On one hand, GEM diphosphate inhibits subunit 1 of ribonucleotide reductase (RRM1), an enzyme involved in normal DNA repair and synthesis. GEM triphosphate competes with the natural nucleotide deoxycytidine triphosphate, for incorporation into DNA during replication, eventually leading to termination of chain elongation and subsequent cell death [2]. Importantly, high levels of expression of RRM1 have been shown to be predictive of resistance to gemcitabine in non-small cell lung cancer (NSCLC) and pancreatic cancer [3,4].

Single-agent GEM showed a good activity and manageable toxicity in advanced breast cancer, with responses as first line treatment ranging from 14% to 37% [5]. Given its non-overlapping toxicity, along with its peculiar mechanism of action, GEM represents an attractive drug to be combined with taxanes in anthracycline-pretreated patients. Moreover, preclinical studies had shown an increase in intracellular concentrations of GEM triphosphate with the sequential administration of paclitaxel (PACL) followed by GEM [6,7], potentially resulting into enhanced antitumor activity. Remarkably, phase II studies of first-line PACL/GEM showed responses in as much as 70% of patients, whereas an activity of 45.5% to 55% was observed when PACL/GEM was used as salvage therapy or in anthracycline-pretreated patients [8,9,10]. Preliminary results of a phase III randomized trial of PACL/GEM versus PACL as first-line treatment in advanced breast cancer patients pretreated with anthracyclines showed a significant advantage in terms of response rate and progression free survival for the combination arm [11]. Interestingly, phase I/II studies suggested that biweekly schedule be one of the best ways of administering GEM in combination with PACL. In fact, with biweekly administration a much higher dose-intensity of GEM can be achieved, thus potentially maximizing the synergy of action between PACL and GEM, as evidenced by some clinical trials [8,12,13,14,15].

Notably, pharmacokinetic studies have shown that the doses needed to optimize the accumulation of GEM triphosphate in mononuclear cells are achieved when GEM is administered at a fixed dose-

rate (FDR) of 10 mg/m²/min [16,17]. As a result, a prolonged infusion time of 10 mg/m²/min may be preferable compared with a standard 30-minute infusion of GEM. To this regard, encouraging results have been observed in phase II trials evaluating FDR GEM either as single-agent or in combination regimens in various solid tumors, including breast cancer [18,19,20,21]. Moreover, a survival advantage was noted for FDR GEM compared with a 30-minute infusion in a randomized phase II study of patients undergoing first-line treatment for advanced adenocarcinoma of the pancreas [22].

Our experience with a biweekly schedule consisting of PACL in combination with a standard 30-minute infusion of GEM in a very heavily pretreated population with advanced breast cancer, showed very favorable preliminary results and a low toxicity profile [15]. For this reason we designed a phase I/II trial of PACL plus FDR GEM, both administered biweekly, in advanced breast cancer patients pretreated with anthracyclines.

PATIENTS AND METHODS

We carried out the phase I of the study enrolling 6 patients in each of 3 steps of escalating doses of FDR GEM (1,000, 1,100, 1,200 mg/m²), in combination with PACL 150 mg/m²; another GEM dose (900 mg/m²) was planned in case of toxicity, but no patient has been enrolled in this step, and 1,200 mg/m² was the recommended dose of GEM for the phase II study.

Forty-two advanced breast cancer patients entered the phase II study. Eligibility criteria included histologically or cytologically confirmed breast cancer pretreated with anthracyclines either in the (neo)adjuvant or advanced setting. To be eligible, patients had to have received at least one line of chemotherapy for advanced disease. In all cases disease progression following the most recent treatment had to be documented. Previous chemotherapy, hormonal therapy and radiotherapy had to be stopped at least 4 weeks prior to study entry. Other eligibility criteria were: presence of evaluable and/or measurable disease, age $\geq$ 18 years, Eastern Cooperative Oncology Group performance status $\leq$ 2, estimated life expectancy $\geq$ 12 weeks and adequate renal, hepatic and hematologic function. Among exclusion criteria there were prior exposure to PACL and/or GEM, presence of symptomatic brain metastases, and history of other malignancy within 5 years of study enrollment with the exception of *in situ* carcinoma of the cervix and adequately treated non-melanoma skin malignancies. Pregnant or nursing women as well as those with reproductive potential not using an effective contraceptive method were excluded from the study. Supportive treatment was at discretion of the investigator. The study protocol was approved by the local Ethic Committee of the Regina Elena National Cancer Institute and written informed consent was obtained from all patients before study enrollment.

Treatment consisted of PACL given at 150 mg/m² as a 3-hour infusion, followed by FDR (10 mg/m²/min) GEM given at 1,200 mg/m² (total infusion time = 120 minutes), both drugs administered on days 1 and 14 of a 28-day cycle. Treatment continued until disease progression, unacceptable toxicity or withdrawal of the patient. Premedication and antiemetic prophylaxis consisted of an antihistaminic, a 5-HT₃ receptor antagonist and dexamethasone given as a 15-minute infusion before chemotherapy. Treatment was postponed by a maximum of 2 weeks if absolute neutrophil count was less than 1,500/mL and/or platelet count was less than 100,000/mL and/or in case of hypertransaminasemia ≥ grade 2. Granulocyte Colony Stimulating Factor (G-CSF) was administered in case of grade 4 neutropenia, and a 25% dose reduction was planned if febrile neutropenia or G3 mucositis occurred. Treatment was discontinued in case of persistent grade 3-4 hypertransaminasemia, neurotoxicity and in any case of cycle delay longer than 3 weeks.

Pretreatment evaluation included medical history and physical examination, hematology and blood chemistry, ECG, chest X-ray, and tumor measurement based on standard radiologic methods or physical examination. During the treatment period hematology and blood chemistry were performed on days 1 and 14 of each cycle. Tumor assessments were performed every 3 cycles and every 3 months during the follow-up period. Response to therapy was assessed according to RECIST criteria [23]. Progression-free survival (PFS) was the time elapsed from the date of initiation of treatment to the date of first evidence of disease progression or death. Overall survival (OS) was the time elapsed from the starting date of treatment to the date of death. Toxicity was graded 1-4 according to NCI-CTC (version 3.0).

In an attempt to identify potential predictive factors of sensitivity to the PACL/GEM combination we retrospectively evaluated the *in situ* protein expression of RRM1 and breast cancer and ovarian susceptibility gene 1 (BRCA1) in primary tumor samples of 15 patients with tissue available for analysis. Tumor samples were evaluated as part of a tissue microarray which included a larger number of advanced breast cancer patients treated with GEM-based chemotherapy at the Regina Elena National Cancer Institute [24]. Analysis of *in situ* protein expression of RRM1 and BRCA1 was done at the Moffit Cancer Centre in Tampa, Florida. Immunofluorescence combined with automated quantitative analysis [AQUA] was used for the *in situ* detection and quantification of RRM1 and BRCA1 proteins as described elsewhere [24].

Statistical consideration

In the phase I of the study, increasing doses of FDR GEM in combination with PACL 150 mg/m², both drugs administered as a biweekly schedule, were given to sequential cohorts of patients including 6 individuals each step. Four steps of different doses of GEM were planned: 1,000 mg/m²; 1,100 mg/m²; 1,200 mg/m²; in the fourth step a dose of 900 mg/m² was planned in case

severe toxicity was experienced in the 1st step (1,000 mg/m²), but no patient required dose reduction to 900 mg/m². Eighteen patients were enrolled in the phase I trial; dose-limiting toxicities (DLT: febrile neutropenia and hyperthrasaminasemia) were evaluated during the first 2 cycles of treatment. At GEM dose-level 3 (1,200 mg/m²), only one hyperthrasaminasemia was found among 6 patients, and therefore this level was chosen for the phase II of the trial.

The primary objective of the phase II trial was response rate. The Simon minimax two-stage design was used to determine the sample size. In the first stage, the regimen would be considered worthy of further testing if at least 5 out of 18 eligible patients had an objective response, with a significance level of 5% and a power of 80%. In the second stage, other 15 patients were needed, with an overall sample size for the phase II of 33 patients. The present analysis is based on data from phase II trial. OS and PFS were estimated by the method of Kaplan and Meyer. Efficacy parameters were evaluated according to an intent-to-treat (ITT) analysis.

RESULTS

Patients characteristics

From November 2004 to December 2007, 42 patients were enrolled in the phase II of the study, all evaluable for activity and toxicity. Patients characteristics are listed in table 1. Median age was 58 years (range 22-78), hormonal receptor status was positive in 35 patients, and 8 patients were HER2 positive, of whom no one had received prior trastuzumab, being all enrolled before 2005 and treated with an anthracycline as first regimen for advanced disease. All patients had been pretreated with anthracyclines either in the (neo)adjuvant or advanced setting, while 21 patients had received prior docetaxel for advanced disease (total patients pretreated with docetaxel n = 22). The median number of previous lines of chemotherapy for advanced disease was 1, although as much as 20 patients had received ≥ 2 prior lines. Thirty patients had viscera as dominant site of disease, while 27 patients had ≥ 2 metastatic sites.

Activity

The median number of administered cycles of PACL/GEM was 6 (range 1-8). Table 2 shows the activity of treatment. Two (4.8%) complete and 14 (33.3%) partial responses were observed, for an overall response rate of 38.1% (95% CI, 23.4%-52.8%). Disease remained stable in 16 patients (38.1%). Responses by disease site were as follows: 100% (3/3) for soft tissue, 22% (2/9) for bone, 37% (11/30) for visceral lesions, with liver metastasis responding in 21% (4/19) of patients. Responses were observed also in 8 of the 20 patients (35%) pretreated with ≥ 2 lines of chemotherapy for advanced disease. Partial responses were also reported in 8 out of the 22 patients (36%) pretreated with docetaxel, as a confirmation of the lack of complete cross-resistance between

PACL and docetaxel. Table 3 shows response by hormonal and HER2 receptor status. No differences were observed between groups, but numbers are very small. Overall, the median PFS was 5 months (95% C.I., 3.4 to 6.6). At a median follow-up of 18 months (range 3-38), the median OS was 19.9 months (95% C.I., 16.2 to 23.6) (Figure 1a,b).

The average scores for RRM1 and BRCA1 protein expression ranged from 610.65-2047.84 and 200-1399.47, respectively. Median values were 1331.42 for RRM1 and 957.78 for BRCA1. For each marker, median values were chosen to divide patients into a high- and low-expression group. No statistically significant association was noted between RRM1 or BRCA1 and response to treatment ($P = 0.4$ for RRM1 and $P = 0.4$ for BRCA1).

Safety

Toxicity of treatment is reported in Table 4. Overall, hematologic toxicity was usually mild to moderate, with severe (grade 3-4) neutropenia occurring in 9.6% of the patients. Neutropenic fever was observed in 5 (12%) patients all of whom received G-CSF at subsequent cycles. Mucositis occurred only in one patient (2.5%) and was grade 1, while nausea/vomiting, mostly of grade 1-2, was recorded in 45.2% of patients. Grade 1-2 sensory neuropathy was encountered in 59.5% of patients, being of grade 3 in only one patient (2.5%). Transient and reversible grade 1-2 hypertransaminasemia occurred in more than half of patients (52.6%). All the 4 patients (9.5%) experiencing a severe hypertransaminasemia had liver metastases at baseline. Fatigue and musculoskeletal pain were related to the total cumulative dose, mostly occurring after the 3rd-4th cycle of treatment. Alopecia was universal. Not any case of cardiotoxicity related to the study drugs, as well as no toxic deaths, were observed. A 25% dose reduction was required in only 1 patient (2.4%), due to grade 4 hypertransaminasemia. Treatment was delayed in 2 patients (4.8%) owing to reversible grade 2 hypertransaminasemia, and was discontinued in 1 patient (2.4%) because of grade 3 sensory neuropathy.

DISCUSSION

Regardless of hormonal and HER2 receptor status, chemotherapy remains an important component of the management of advanced breast cancer patients. The “optimal” treatment of pretreated patients depends on a number of variables, such as molecular issues, sites of disease and previous therapy. Particularly, for patients with HER2-negative advanced breast cancer there is an urgent need of innovative and effective anthracycline-free combination regimens. Also, improved activity of chemotherapy may derive from scheduling variation, in order to better exploit pharmacokinetic and pharmacodynamic interactions of the drugs employed. The use of a combination of a taxane plus gemcitabine is among the best treatment options for HER2-negative advanced breast cancer

patients who have been pretreated with anthracyclines [25,26,27]. Although phase III comparisons between PACL/GEM and docetaxel/GEM are lacking, a small randomized phase II study suggested that PACL be the best taxane to be combined with GEM on account of a safer toxicity profile of PACL/GEM compared with docetaxel/GEM [28].

In the present trial, FDR GEM in association with PACL produced a response rate of 38.1%, which is slightly inferior to the 45.5% to 55% of responses reported with the same combination in phase II studies of pretreated patients [25]. Several reasons could be put forward in order to explain this finding. Firstly, more than 70% of patients in our trial had visceral disease and nearly half of all patients had been pretreated with ≥ 2 lines of chemotherapy for advanced disease. Secondly, the population of our trial included 19% of patients with HER2-positive breast cancer, a disease entity known to be associated with a particularly aggressive clinical course in the absence of treatment with anti-HER2 drugs such as the monoclonal antibody trastuzumab [29]. Moreover a pharmacokinetic study evaluating FDR GEM with or without PACL in patients with NSCLC, suggested the presence of inter- and intra-patients variability in the plasmatic concentrations of GEM [30]. Such a finding suggests that individualization of the dose rate could be crucial in order to achieve effective dosing of GEM. More recently, a randomized crossover study of GEM 1,000 mg/m² given as a 30-minute versus a FDR infusion at 10 mg/m²/min carried out in 33 patients, showed that intracellular accumulation of GEM was greater with the prolonged infusion, but there was an increase in GEM-triphosphate accumulation between weeks 1 and 2, indicating a self-induction of GEM accumulation [31]. Also, a significant heterogeneity of GEM triphosphate was noted, thus suggesting that the optimal dose rate needed to optimize intracellular accumulation of GEM triphosphate may change overtime, and a fixed dose rate of 10 mg/m²/min could not be optimal for all patients and for the same patient over time [31]. Nevertheless, despite all these considerations, in the present study FDR GEM plus PACL given biweekly was not able to improve the remarkable response rate of 53% observed in final results of our previous experience with the use of a similar schedule of PACL/GEM with GEM given as a standard 30-minute infusion [32].

Importantly, the identification of women who will benefit from palliative chemotherapy would allow physicians to deliver effective treatments to sensitive patients, while preventing others from suffering the side effects of inactive drugs. Unfortunately, in this trial we did not observe any significant correlation between response to treatment and RRM1 or BRCA1, two biomarkers which could predict sensitivity to GEM and taxanes, respectively [3,33]. However, only 15/42 patients in the present trial had tissue available for analysis of RRM1 and BRCA1, and this small sample should be regarded as one of the reasons for the negative results observed. Nevertheless the search for biomarkers that could predict response to treatment should be pursued actively in future clinical trials of breast cancer. To date, estrogen receptor (ER)- α , progesterone receptor (PgR) and HER2

represent the only biomarkers used in clinical practice to aid treatment decisions in both early and advanced breast cancer.

Importantly, we observed a very favorable toxicity profile for FDR GEM plus PACL, especially for hematologic toxicity. Also, toxicity was manageable and reversible with few treatment delays. Of note, only one patient had to discontinue treatment owing to toxicity, namely a grade 3 sensory neuropathy related to PACL. The low toxicity observed in the present trial confirm that dose intensification obtained by prolonging GEM infusion is safe in advanced breast cancer [21]. Interestingly, the good safety profile of FDR GEM might not apply to all cancers, particularly in NSCLC, in which FDR GEM, given as single-agent in patients not eligible for platinum-based chemotherapy, was found to be associated with an unacceptable rate of hematologic toxicity compared with a standard 30-minute infusion [34].

In conclusion, FDR GEM plus PACL showed to be active and safe in advanced breast cancer patients pretreated with anthracyclines. However, the prolonged GEM infusion did not seem to offer a clinical advantage over a standard infusion regimen, according to other literature results failing to demonstrate an advantage in various solid tumors [35,36,37]. Importantly, following the positive results of antiangiogenic therapies for HER2-negative breast cancer [38], the association of a taxane/GEM doublet with the anti-VEGF agent bevacizumab is currently one of the most appealing strategies, and several trials are being conducted in order to evaluate this three-drug regimen [39,40].

Conflict of interest statement. The authors indicated no potential conflicts of interest.

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Table 1. Patient characteristics

Characteristic	No. of pts
Entered/evaluable	42 / 42
Median age (range)	58 (22-78)
Median performance status (range)	0 (0-2)
Premenopausal	6/36
Hormonal receptor status (ER and/or PgR)	
Positive	35
Negative	7
HER2 status	
Positive	8
Negative	25
Unknown	9
Prior hormonal treatment (adjuvant/advanced)	35 (20/34)
Prior (neo)adjuvant chemotherapy (anthracycline/docetaxel)	25 (11/1)
Prior chemotherapy for advanced disease	
Anthracycline	16
Docetaxel	21
Anthracycline and docetaxel	15
Other cytotoxics	5
Median no. of prior lines of chemotherapy for advanced disease (range)	1 (1- 4)
Dominant metastatic site	
Soft tissue	3
Bone	9
Viscera (liver)	30 (19)
No. of disease sites	
1	15
2	23
3	4

Table 2. Activity of treatment

	N°	%	
CR	2	4.8	} 38.1 % (95% CI, 23.4%-52.8%)
PR	14	33.3	
NC	16	38.1	
PD	10	23.8	

Table 3. Response to treatment by hormonal receptor and HER2 status

	N°	%
ER and/or PgR +	13 / 35	37.1
ER and/or PgR -	3 / 7	42.8
HER-2 +	4 / 8	50.0
HER-2 -	9 / 25	36.0

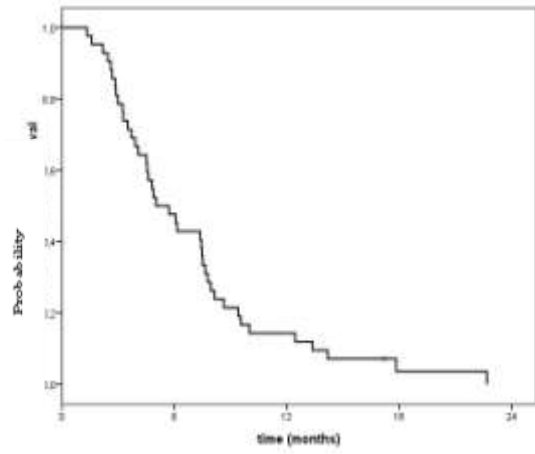
Table 4. Summary of treatment-related adverse events in 42 patients

Toxicity	Grade (%)		
	1-2	3	4
Neutropenia	18 (42.8)	2 (4.8)	2 (4.8)
Febrile neutropenia	-	3 (7.1)	2 (4.8)
Thrombocytopenia	9 (21.4)	0 (0)	0 (0)
Anemia	29 (69)	1 (2.4)	0 (0)
Mucositis	1 (2.4)	0 (0)	0 (0)
Nausea/vomiting	18 (42.8)	1 (2.4)	0 (0)
Sensory neuropathy	25 (59.5)	1 (2.4)	0 (0)
Alopecia	5 (11.9)	26 (61.9)	0 (0)
Hypertransaminasemia	22 (52.6)	3 (7.1)	1 (2.4)
Fatigue	14 (33.3)	1 (2.4)	0 (0)
Musculoskeletal pain	10 (23.8)	0 (0)	0 (0)

Legend of Figure:

Fig. 1: Progression free survival (a) and overall survival (b) in 42 enrolled patients

a



b

