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Mitomycin C and Capecitabine Combination (MiXe) in Heavily Pretreated Metastatic Breast Cancer Patients. A Dose-finding Study

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Abstract. *Background: No standard chemotherapy has been defined for metastatic breast cancer patients pretreated with anthracyclines and taxanes. In preclinical studies, mitomycin C (MMC) and capecitabine showed a synergistic effect by up-regulation of thymidine phosphorylase, and both drugs were active against breast cancer with a lack of overlapping toxicity, making their combination a well-tolerated regimen. Patients and Methods: A dose-finding study was carried out in order to determine the maximum tolerable dose of MMC combined with fixed-dose capecitabine and to describe the dose-limiting toxicities. Results: Twenty-one patients were enrolled, with metastatic breast cancer pretreated at least with anthracyclines and taxanes (3 at dose level I, 15 at dose level II, 3 at dose level III). At dose level III (MMC 12 mg/m^2 and capecitabine 1000 mg/m^2 days 2-15) dose-limiting toxicities were recorded in 2 patients (G4 thrombocytopenia, neutropenic fever, G4 neutropenia); dose level II (MMC 10 mg/m^2 and capecitabine 1000 mg/m^2 days 2-15) was extended for a better safety evaluation. No severe toxicity was noted at this dose level, and therefore this dose was recommend for the phase II study. With regard to activity, 4 partial responses and 2 stable diseases (28%) were recorded. Conclusion: Our data show that the combination is feasible, well tolerated and active in this set of patients.*

Anthracyclines and taxanes are considered the most effective drugs against breast cancer, but their early use in the treatment of these tumors has led to an increase in the number of patients with disease resistance to both agents. Therefore, new therapeutic strategies are needed for these patients, in which the major aims of treatment are to obtain disease control, symptom reduction and better quality of life. Several drugs, alone or in combination, have been tested in this stage of disease, and response rates ranging from 0% to 62% have been reported in small phase II studies or in retrospective analyses (1).

Capecitabine has become an important treatment option for patients with metastatic breast cancer (MBC) that has progressed following taxane therapy, and has shown promise in anthracycline-pretreated patients, as monotherapy and as a component of combination regimens (2-6). Capecitabine is an oral fluoropyrimidine that undergoes conversion to its active metabolite fluorouracil (5-FU) through a 3-step enzymatic metabolic process (7). The final step of this process, the conversion of 5'-deoxy-5-fluorouridine (5'-DFUR) to 5-FU, requires thymidine phosphorylase (dThdPase), a potent tumor-associated angiogenesis factor. The higher expression of dThdPase in tumor tissue provides for preferential conversion of capecitabine in neoplastic tissues (8). Given the prominent role of dThdPase in the therapeutic index of capecitabine-based treatment, it follows that maximizing dThdPase activity would result in an enhanced therapeutic index. Treatment of malignant tumors with various cytokines, such as tumor necrosis factor alpha, interleukin-1 and interferon gamma has been observed to produce increases in intratumoral dThdPase activity and to enhance tumor sensitivity to 5'-DFUR *in vitro* and *in vivo* (9). In addition, paclitaxel (100 mg/kg), docetaxel (15 mg/kg) and mitomycin C (MMC) (5 mg/kg) have also been shown to increase the levels of dThdPase in human colon cancer xenograft studies by 8-fold, 6.1-fold, and 7.7-fold, respectively (10). These cytotoxic agents are thought to up-regulate dThdPase through increases in tumor necrosis factor alpha levels.

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MMC has been a treatment option for MBC since the early 1980s (11). It belongs to the family of antitumor antibiotics, and also works against hypoxic tumor cells (12). Its toxicity is comparable to that of other cytotoxic agents, consisting of neutropenia and thrombocytopenia. Chronic toxicities, such as renal failure due to the development of hemolytic uremic syndrome (HUS) or pulmonary toxicity, are infrequent but may be severe and, therefore, the upfront use of MMC should be avoided. The combination of MMC with 5-FU and folinic acid was used in MBC with interesting results (13). On account of preclinical data, the mechanisms of action and lack of overlapping toxicities of MMC and capecitabine, we conducted a dose-finding study of this combination in MBC previously treated with anthracyclines and taxanes. The study was designed to determine the maximum tolerable dose (MTD) of MMC combined with fixed-dose capecitabine and to describe the dose-limiting toxicities (DLTs) of the combination.

Patients and Methods

Eligibility. Patients with cyto/histologically–confirmed MBC pretreated with anthracyclines and taxanes were eligible for this study. Bidimensionally measurable disease was not a prerequisite, but patients received a complete evaluation of their sites of disease and each measurable lesion was recorded. Eligibility criteria also included the following: (i) age ≥ 18 years; (ii) ECOG performance status of 0 to 3; (iii) a life expectancy ≥ 12 weeks; (iv) no major surgery, radiotherapy, or chemotherapy within 28 days of study entry; (v) no prior MMC or capecitabine treatment; (vi) no gastrointestinal disorder that might affect the absorption of capecitabine; (vii) adequate hematopoietic WBC count ≥ 3,000/mm^3, absolute neutrophil count (ANC) of ≥ 1,500/mm^3, platelet count ≥100,000/mm^3 and hemoglobin level of ≥ 9.0 g/dL; (viii) hepatic (total serum bilirubin level ≤ 1.5 times institutional upper normal limits [UNL], AST and ALT ≤ 3 times UNL) and renal (serum creatinine ≤ 1.5 times UNL or calculated creatinine clearance ≥ 60 mL/min) functions; (ix) no brain metastases, unless the lesions had been previously irradiated and were stable and asymptomatic; (x) absence of organ allografts or serious uncontrolled infections; (xi) no known existing coagulopathy or requirement for therapeutic doses of coumarin-based anticoagulants; and (xii) no history of severe reaction to fluoropyrimidine therapy. Patients gave written informed consent before treatment.

Treatment plan. Capecitabine was administered at the fixed dose of 2000 mg/m^2/day in two equally divided oral doses of 1000 mg/m^2 for 14 days starting on days 2 through 15 every 21 days. MMC was planned to be administered at 4 escalating dose levels of 7 mg/m^2 (level 1); 10 mg/m^2 (level 2); 12 mg/m^2 (level 3) and 14 mg/m^2 (level 4) every 42 days by *i.v.* infusion. A minimum of 3 new patients were to be treated at all tolerable dose levels. Intrasubject dose escalation was not permitted. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC). DLT was defined as one of the following: (i) an ANC less than 500/mm^3 for more than 5 days, or associated with fever (temperature ≥ 38.5˚C); (ii) a platelet count less than 25,000/mm^3; (iii) severe (≥ grade 3) non-hematological toxicity (except alopecia) that resulted in interruption of capecitabine for more than 3 days, or delay in the dose of MMC for more than a week; and (iv) clinical inability (because of toxicity) to start the next cycle of treatment within 2 weeks of the planned start date. The maximum-tolerated dose level was defined as the highest MMC dose level which, when combined with capecitabine 2000 mg/m^2 day, resulted in DLT in less than 2 out of 6 new patients, during both their first and second cycles of treatment. An observation period of 6 weeks (2 cycles of chemotherapy) for the development of DLTs was required by the protocol.

Dosage modifications. A new course of treatment was to begin only when the granulocyte count was ≥ 1,500/mm^3 and the platelet count was ≥ 100,000/mm^3 and any other treatment-related toxicities were ≤ grade 1; otherwise, treatment was withheld for up to 1 week. If the toxicity had not resolved from grade 0 to 1 at the end of this period, the patient was withdrawn from the study unless clinical benefit had been documented, in which case a treatment delay of up to 1 additional week to allow recovery was permitted. Planned treatment with capecitabine within a cycle of therapy was withheld in the presence of grade ≥ 2 non-hematological (except isolated-hyperbilirubinemia or alopecia) or grade ≥ 3 hematological toxicity. Treatment was resumed when

Table I. *Patient characteristics.*

	No. of patients
Enrolled	21
Age (years)	
Median	61
Range	42-77
ECOG Performance Status	
0	4
1	8
2	8
3	1
Prior chemotherapy	
Adjuvant	17
Metastatic	21
Anthracycline and taxane	21
Vinorelbine	9
5-Fluorouracil bolus	9
Gemcitabine	3
Cisplatin	2
1 Line	4
2 Lines	12
3 Lines	3
4 Lines	2
Site of metastases	
Liver	11
Lung	10
Bone	10
Lymph nodes	4
Skin	4
1 Site	4
2 Sites	9
3 Sites	7
4 Sites	1

Table II. *Toxicity per dose level in cycles one and two according NCI-CTC.*

Dose level	Patients	Neutropenia 1 2 3 4	Neutropenic fever	Thrombocytopenia 1 2 3 4	HFS 1 2 3	Nausea/vomit 1 2 3 4	Stomatitis/mucositis 1 2 3 4	Diarrhea 1 2 3 4
1	3	1			1		1	1
2	15	2		1		1		
3	3	1 1	1	1				2

Table III. *Summary of adverse events for the patients at dose level 2 (all cycles).*

Patients	Neutropenia 1 2 3 4	Neutropenic fever	Thrombocytopenia 1 2 3 4	HFS 1 2 3	Nausea/vomit 1 2 3 4	Stomatitis/mucositis 1 2 3 4	Diarrhea 1 2 3 4
15 (I/II cycle)	2		1		1		
10 (III/IV cycle)	1 1	1	1	2 1	1	1	1
8 (V/VI cycle)	2 2		2	2	2	2	2
4 (VII/VIII cycle)	2		1	1 1	2	1 1	1

hematological or non-hematological toxicity, except hand-foot syndrome (HFS), resolved from grade 0 to 2. In such cases, capecitabine was resumed at either the original dose level for grade 2 non-hematological toxicity and grade 3 hematological, or with a 25% of reduction for grade ≥ 3 non-hematological or grade 4 hematological toxicity. For grade 2 to 3 HFS, capecitabine treatment was withheld until resolution to ≤ grade 1 and then restarted at the same dose (for grade 2 HFS) or at the preceding dose level (for grade 3 HFS). Capecitabine treatment was not interrupted for isolated hyperbilirubinemia.

Baseline and treatment evaluations. Histories, physical examinations and routine laboratory studies were performed before treatment and weekly thereafter. Routine laboratory studies included serum electrolytes, chemistries, complete blood cell counts with differential WBC counts, blood clotting times and urinalysis. Toxicity was graded according to NCI-CTC. Tumor assessment was made by TC and bone scan. Tumors were measured after every 2 courses, and treatment was continued in the absence of progressive disease or intolerable toxicity. A complete response was defined as the disappearance of all disease on 2 measurements separated by a minimum of 4 weeks. A partial response required more than 50% reduction in the sum of the products of the bidimensional measurements of all measurable lesions documented by 2 measurements separated by at least 4 weeks, and progressive disease required an increase in 25% in the sum of the products of the bidimensional measurements of all measurable lesions or the appearance of new lesions.

Results

A total of 21 patients were enrolled between April 2003 and September 2004. All patients were evaluable for safety, and their characteristics are provided in Table I. The majority of patients (17) had an ECOG performance status ≥ 1. The liver, lung and bone were the most common metastatic sites. Seventeen patients had received ≥ 2 lines of chemotherapy including anthracyclines and taxanes, and 9 patients had been pretreated with bolus 5-FU regimens. Three patients were enrolled at dose level I, 15 at dose level II and 3 at dose level III. A total of 121 cycles of chemotherapy were delivered. None of the patients treated at dose level I developed DLTs. The first 3 patients at dose level II did not develop DLTs, even if 1 patient had G2 thrombocytopenia. At dose level III, 2 patients reported DLTs. Specifically, 1 patient reported G4 thrombocytopenia and neutropenic fever and 1 patient G4 neutropenia. For this reason, dose level II accrual was extended. At this dose level, 15 patients were treated and received a total of 64 cycles (range 2-8). Two patients developed DLTs: neutropenic fever 1 patient and HFS G3 1 patient. In both cases, toxicities broke out at the fourth cycle. The dose intensity was calculated for all patient dose levels; a median dose intensity of 100% was delivered during cycle 1 and 2 for both drugs. Eleven patients received the third and

fourth cycles of treatment; 5 out of 11 patients had therapy delayed, 3 for hematological toxicity (G2 neutropenia) and 2 for G2 HFS. The dose intensity delivered during cycles 3 and 4 was 75% for MMC and 84% for capecitabine. Four patients completed all 8 planned courses of chemotherapy (4 MMC and 8 capecitabine).

Safety. Hematological and non-hematological adverse events during the first and second cycle (the time period for the evaluation of DLTs) are summarized in Table II. The toxicities recorded for 15 patients treated at dose level II are provided in Table III. No toxic death occurred. No DLT occurred at dose I or in the first 3 patients at dose level II. At dose level III, 2 out of 3 patients reported DLTs. One patient had G4 thrombocytopenia and neutropenic fever, and 1 patient reported G4 neutropenia. Among 15 patients enrolled at dose level II, no one developed DLT during the first and second cycle. Ten out of 15 patients received cycles 3 and 4. In this group, 2 patients reported DLTs: neutropenic fever in 1 patient and HFS G3 in 1 patient. In both cases, toxicity broke out at the fourth cycle, after 2 MMC administrations at a cumulative dose of 20 mg/m^2. Other recorded toxicities were: 3 episodes of G3 neutropenia, 3 episodes of G2 thrombocytopenia, without bleeding. Two patients suffered from G2 HFS and 4 had mild HSF. No evidence of MMC-induced hemolytic-uremic syndrome was documented.

Activity. All patients completed at least 2 cycles of chemotherapy and were evaluable for activity. All patients were pretreated with anthracyclines and taxanes, 15 were also pretreated with gemcitabine, vinorelbine and bolus 5-FU, cisplatin. We recorded 4 partial responses (20%) and 2 stable diseases, lasting ≥ 16 weeks. Seven patients progressed at the first evaluation.

Discussion

No standard chemotherapy has been defined for MBC in progression after treatment with anthracyclines and taxanes, and these heavily pretreated patients need additional effective chemotherapy. Capecitabine, as a single agent, has been shown to be effective and well tolerated in this population (2-6). Preclinical studies showed that, in malignant tissues, dThdPase is increased compared to normal tissues (7); this enzyme mediates the conversion of capecitabine into the active compound 5-FU. It follows that strategies directed to enhance the expression of dThdPase may improve capecitabine activity. Sawada *et al.* (10) demonstrated that some drugs, such as taxanes and MMC, enhanced the expression of dThdPase in human tumor xenografts. MMC is an active drug against breast cancer, with a response rate of 15-20% in previously treated MBC. The main side-effect is bone marrow suppression, which is dose-related and delayed. Therefore, the combination of MMC and capecitabine should be favorable since they have a synergistic effect, different mechanisms of action and different toxicity profiles.

In our study, all patients were heavily pretreated and had poor prognosis. In this stage of disease, the aims are the control of disease and the improvement of quality of life. Our data indicate that MMC at dose of 10 mg/m^2 on day 1 every 6 weeks and capecitabine 1000 mg/m^2 twice daily on days 2-15 every 3 weeks is safe, with an adverse events profile similar to capecitabine monotherapy. At the dose of MMC 12 mg/m^2, hematological DLTs occurred in 2 out of 3 patients related to MMC. On the other hand, among the 15 patients enrolled at dose level II, none reported DLT in the first 2 cycles. For the 10 patients who received cycles 3 and 4, we recorded DLTs in 2 patients (neutropenic fever in 1 patient and HFS G3 in 1 patient). We also registered 3 episodes of G3 neutropenia, 3 episodes of G2 thrombocytopenia and 2 patients suffered from G2 HFS. In all cases, adverse events broke out at the fourth cycle after a cumulative dose of MMC 20 mg/m^2. The dose intensity during the first 2 cycles was maintained at 100% for both drugs, with a reduction of 25% for MMC and 16% for capecitabine in cycles 3 and 4. Despite the fact that all patients were heavily pretreated, hematological toxicity was moderate and was fully recovered with 1 week of rest. In this study, the incidence of HFS was low, probably linked to the capecitabine dose of 2000 mg/m^2 daily, as reported by other authors(14).

With regard to activity, we recorded tumor growth control (4 PR and 2 SD) in 6 patients (28%). The activity and favorable safety profile of capecitabine in MBC prompted several clinical trials in order to confirm the early data in pretreated MBC. Capecitabine single agent was evaluated as first-line chemotherapy, mostly in elderly patients, and was shown to be active with mild toxicity (14,15). In the randomized study by O'Shaughnessy *et al.* (16), the combination of capecitabine and docetaxel compared to docetaxel alone showed superior survival time, TTP and response rate, with manageable toxicity. Recent phase I - II studies tested the combination of vinorelbine and capecitabine in pretreated or elderly MBC patients. In pretreated patients, the authors recorded a response rate ranging from 50% to 55% with an incidence of G3-4 neutropenia in 29% - 39% of the patients (17,18). In elderly patients, the combination gave a response rate of 50%, neutropenia being the main toxicity (19). Our data show that the combination of MMC and capecitabine at dose level II is well tolerated with only 3 episodes of G3 neutropenia and 1 episode of febrile neutropenia.

MMC-capecitabine was also employed in gastrointestinal tumors. Hofheinz *et al.* (20), in an extended phase I trial

based on pretreated patients with gastrointestinal cancers, found that the recommended doses was MMC 10 mg/m^2 and capecitabine 1000 mg/m^2 twice daily on days 1 – 14 every 3 weeks. At these doses the side-effects were moderate without G4 events. Rao *et al.* (21), in a phase II study focused on elderly patients with advanced colorectal cancer, reported a response rate of 38% with a favorable toxicity profile. In a randomised phase II study in patients with advanced biliary tract cancer, Kornek *et al.* (22) compared MMC in combination with capecitabine or biweekly high-dose gemcitabine. The authors concluded that both combination regimens are feasible, tolerable and clinically active, even if MMC- capecitabine seems to be more effective. In conclusion, efficacious chemotherapies are needed in anthracycline- and taxane-pretreated MBC patients. Capecitabine has shown to be an active and well-tolerated drug in this population, thus it seems to be an ideal drug to test new combination chemotherapies, such as in our dose-finding study. A phase II study with MMC 10 mg/m^2 every 6 weeks and capecitabine 1000 mg/m^2 twice daily on days 2-15 every 3 weeks is currently ongoing.

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