Clinical Trial Report
Future Oncology

Safety and therapeutic effect of metronomic chemotherapy with cyclophosphamide and celecoxib in advanced breast cancer patients

Herman A Perroud‡[1], Maria José Rico‡[1], Carlos M Alasino[2], Francisco Queralt[2], Leandro E Mainetti[1], Stella M Pezzotto[3], Viviana R Rozados[1] & O Graciela Scharovsky*[1]

[1]Institute of Experimental Genetics, School of Medical Sciences, National University of Rosario, Argentina
[2]Rosario Institute of Oncology, Rosario, Argentina
[3]Institute of Immunology, School of Medical Sciences, National University of Rosario, Argentina
*Author for correspondence: Tel.: +54 341 480 4558/63 ext: 244 ■ Fax: +54 341 480 4569
■ graciela.scharovsky@gmail.com
‡Authors contributed equally

Metronomic chemotherapy (MCT), the chronic administration, at regular intervals, of low doses of chemotherapeutic drugs without extended rest periods, allows chronic treatment with therapeutic efficacy and low toxicity. Our preclinical results suggested that combined MCT with cyclophosphamide and celecoxib could inhibit breast cancer growth. The aim of this study was to determine the toxicity, safety and efficacy of oral MCT with cyclophosphamide 50 mg *per orem* daily and celecoxib 400 mg (200 mg *per orem* two-times a day) in advanced breast cancer patients. During the first stage of the study, the therapeutic response consisted of prolonged stable disease for ≥24 weeks in six out of 15 (40%) patients with a median duration of 37.5 weeks and a partial response in one out of 15 (response rate: 6.7%) patients lasting 6 weeks . The overall clinical benefit rate was 46.7%. The median time to progression was 14 weeks. Progression-free survival at 24 weeks was 40% and the 1-year overall survival rate was 46.7%. The adverse events were mild (gastric, grade 1; and hematologic, grade 1 or 2). No grade 3 or 4 toxicities were associated with the treatment. Evaluation of patients' quality of life showed no changes during the response period. MCT with cyclophosphamide plus celecoxib is safe and shows a therapeutic effect in advanced breast cancer patients.

Breast cancer is the most common cancer and the second leading cause of cancer death in American women, exceeded only by lung cancer [1]. In 2008 there were 104,859 new cases of malignant tumors in Argentina, breast cancer being the malignant illness with the highest incidence in women (74 out of 100,000 women) [2].

Conventional cancer therapies (surgery, radiotherapy and chemotherapy) seem to have reached a therapeutic efficacy plateau with relative toxicity and detriment to the patients' quality of life. These characteristics account for the increased costs in public health due to the need to implement compensatory treatments and hospital admissions.

In 2000 there was a turning point in cancer chemotherapy, with four important papers published in a period of 2 months. Browder *et al.* demonstrated the antiangiogenic effect of cyclophosphamide (Cy) using a low-dose schedule; they also found that this effect was lost when using high doses and standard schedules because of tumor vasculature re-growth [3]. Klement *et al.* demonstrated regression of large established tumors without an ensuing increase in host toxicity using low doses of vinblastine [4]. This new modality of drug administration was named as 'metronomic' by Hanahan *et al.* [5]. Thus, metronomic chemotherapy (MCT) was defined as the administration of chronic equally spaced and, generally, low doses of chemotherapeutic drugs without extended rest periods, which allow for chronic treatment with therapeutic efficacy and low toxicity. Those papers together with that by Fidler and Ellis stating that "Cancer is a chronic disease and should be treated like other chronic diseases" launched MCT research [6].

At variance with what is observed when using standard chemotherapy schemes, Browder *et al.* and Klement *et al.* showed that MCT avoided

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drug resistance [3,4]. This may be attributed to the high-genetic stability of endothelial cells, the main target of MCT, making them less prone to developing drug resistance [7]. However, this concept was challenged by the demonstration, in a preclinical study, of the development of resistance to MCT, although due to different mechanisms than those involved in resistance to drugs administered in the maximum-tolerated dose [8].

Tumor angiogenesis is a tumor microenvironmental process that promotes tumor cell survival and growth, invasion and metastasis [9]. Hence, antiangiogenic therapies should also serve as anti-tumor ones.

Several mechanisms account for the antiangiogenic effect of MCT observed *in vivo*, including selective inhibition of proliferation and/or induction of apoptosis in activated endothelial cells, selective inhibition of endothelial cell migration, increased expression of the endogenous angiogenesis inhibitor thrombospondin-1 (TSP-1) and a sustained decrease in levels and viability of bone marrow-derived endothelial progenitor cells [10]. However, inhibition of angiogenesis is not the exclusive mechanism of action of MCT since certain drugs, such as Cy, may contribute to the efficacy of the treatment through the stimulation of the immune response [10–12]

Cy, an alkylating drug that exerts a toxic effect on proliferating cells, is one of the most used drugs in cancer chemotherapy, being one of the earliest cytotoxic drugs used in MCT [3].

Cyclooxygenase 2 is a prostaglandin synthase enzyme that has been implicated in multiple stages throughout the tumorigenic process and is overexpressed in a variety of malignancies including breast cancer [13]. This is in contrast with cyclooxygenase 1, which is constitutively expressed in normal tissues. Recently, elevated cyclooxygenase 2 expression in breast cancer has also been associated with the presence of distant metastases [14]. Celecoxib (Cel) is a selective inhibitor of cyclooxygenase 2 that has shown antiangiogenic [15] and antiproliferative [16] properties in breast cancer cell lines.

The anti-tumor and antimetastatic properties of MCT with Cy as a monotherapy [17] or in combination with Cel [18] were demonstrated in our preclinical tumor models. The promising results obtained, in terms of therapeutic efficacy, good tolerability and low-toxicity profile, together with previous clinical experience by others setting the metronomic dose of Cy [19,20] prompted us to conduct a Phase II trial employing metronomic administration of Cy combined with Cel for the treatment of metastatic breast cancer patients progressing after standard chemotherapy.

In addition, we evaluated several biomarkers of angiogenesis, including serum concentrations of VEGF and TSP-1, and circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs), in an attempt to define their ability to predict therapeutic response.

Patients & methods

Eligibility

Recruited patients were required to have histologically confirmed advanced breast cancer progressing after three and no more than four chemotherapy schemes. Other inclusion criteria were: aged between 18 and 80 years; adequate bone marrow, renal and liver function; normal calcemia; at least one lesion according to Response Evaluation Criteria In Solid Tumors; and more than 3 months of life expectancy. It was mandatory that all patients must have recovered from any prior chemotherapy, radiotherapy or surgery before their inclusion. Each patient included in this study gave her written informed consent. This study was authorized by the School of Medicine Bioethics Committee and by the Argentine regulatory agency (ANMAT).

Treatment & evaluation

All patients received Cy 50 mg *per orem* daily, plus Cel 400 mg (200 mg *per orem* two-times daily).

Baseline evaluation included clinical examination, chest X-ray, liver ultrasound or CT scan, and complete biochemical and hematologic tests. Patients were evaluated by physical examination and hematologic tests every 14 days during the first 6 months of treatment and every 28 days thereafter.

Clinical response and toxicity were evaluated every 2 months, or earlier if it was necessary. Patients were followed until progression or death.

In case of grade 2 neutropenia or thrombocytopenia, the dose of Cy was reduced to 50% (25 mg/day), while if grade 3 toxicity was reached it was reduced to 25% of the original dose (12.5 mg/day). In the case of grade 4 toxicity, treatment was discontinued until hematologic recovery.

In case of nausea and vomiting, dyspepsia or grade 3 abdominal pain, Cel was reduced to 50% (200 mg/day). The study was discontinued if there were more than two dose reductions or any gastrointestinal bleed grade 2 or more.

Evaluation of CECs & CEPs

Quantification of CECs and CEPs by flow cytometry was carried out on peripheral blood collected in tubes with ethylenediaminetetraacetic acid and then separated by Ficoll–Hypaque gradient. Samples were collected at baseline, at the first three visits and every 2 months thereafter. A panel of monoclonal antibodies, including anti-CD45-FITC (BD Pharmingen, CA, USA) to exclude hematopoietic cells, anti-CD31-ALEXA Fluor® 488 (BD Pharmingen) and anti-CD133-PE (Miltenyi Biotec, Cologne, Germany), were used to determine the percentages of CECs and CEPs, respectively. Samples were evaluated using an Epics® XL™ (Beckman Coulter, High Wycombe, UK) flow cytometer.

VEGF & TSP-1 quantification

Levels of VEGF and TSP-1 were evaluated by ELISA in serum samples collected at baseline, at the first three visits and every 2 months thereafter with VEGF Quantikine® (R&D Systems, MN, USA) and TSP-1 Quantikine kits. Blood samples were allowed to clot for 2 h at room temperature. After centrifugation, the serum was removed and stored at -20°C until used. The ELISA method was performed in duplicates as according to the manufacturer instructions.

Adverse events & quality of life

Toxicity was evaluated according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0. Quality of life was evaluated by the Eastern Cooperative Oncologic Group scale (ECOG), Brief Pain Inventory [21] and Functional Assessment of Cancer Therapy Breast (FACT-B) questionnaires. Patients' presence of pain, pain intensity, pain relief, physical well being, social/family well being, functional well being, emotional well being and additional concerns were analyzed at three time points: baseline; middle; and end of treatment.

Study end points

The primary end point was the overall clinical benefit (OCB), which was defined as complete response (CR) plus partial response (PR) and prolonged stable disease (pSD) ≥24 weeks. Progressive patients were those who did not show CR, PR or pSD. Nonprogressive patients were those who showed any CR, PR or pSD. Tumor assessments were performed every 2 months according to WHO standard criteria and the response was evaluated by Response Evaluation Criteria in Solid Tumors. Safety and tolerability were determined on the basis of the presence and grade of adverse events. Time to progression (TTP) was calculated from baseline to the first documentation of progressive disease or related death, if this occurred before progressive disease. Patients with death unrelated to breast cancer or this treatment were censored in TTP analysis. Progression-free survival (PFS), response rate (RR) and overall survival (OS) were also calculated.

Study design & statistical analysis

For this nonrandomized Phase II trial, sample size calculation was based on an optimal two-stage minimax design [22]. This approach was used to investigate whether the overall RR is sufficient to proceed to Phase III trials with a minimum number of patients. The sample size for the trial was determined as follows. The threshold RR was defined at 5% and the expected RR was set at 25%. This study was regarded to be adequate to recruit a total of 25 patients (15 in the first stage and an additional ten in the second), assuming an α-error of 0.05 and β-error of 0.10 based on Simon two-stage Phase II design [23]. The decision to continue or not to the second stage is based on the RR obtained in the first stage. If any response is obtained in at least one patient, the second stage will be carried out. The evaluation of biomarkers was a secondary goal. Hence, the sample size was calculated for the main objective, that is to say, evaluation of safety and efficacy of the treatment.

The results were statistically analyzed, depending on the variable distribution, using parametric or nonparametric tests. Friedman and Wilcoxon signed-rank tests were used to assess whether median scores of FACT-B items were significantly different from baseline. Variations in VEGF and TSP-1 serum levels during treatment were evaluated by the linear regression test. Kaplan–Meier analysis was used to evaluate PFS and OS. Data were analyzed using the STATA statistical software (version six) [24].

A multivariate logistic regression analysis was performed in order to evaluate the association of hormonal status (patients with negative estrogen receptor and progesterone receptor vs patients with at least one positive receptor) and baseline ECOG status (ECOG >2 vs ECOG ≤2) to the response. Age-adjusted odds ratios were calculated.

Results

Patient characteristics

Fifteen patients were enrolled, thus concluding the first stage of the study, while the enrollment of patients continues until completion of the

Table 1. Patient characteristics.

Characteristic	Patients (n)
Median age (range)	
At diagnosis (years)	51 (36–72)
At the beginning of MCT (years)	61 (38–78)
Menopausal status	
Premenopausal	1
Postmenopausal	14
Metastases location	
Lung	7
Hepatic	7
Skin/soft tissue	5
Bone	11
Brain	2
Others	3
Number of metastases	
1	3
2	8
>2	4
Previous treatment	
Surgery (n = 15)	14
Radiotherapy (n = 15)	12
Number of previous chemotherapy lines	
3	8
4	7
ECOG performance status	
1	4
2	8
>2	3
ER status	
Positive	9
Negative	6
PgR status	
Positive	6
Negative	9
Her2/Neu status	
Positive	6
Negative	7
Unknown	2

ECOG: Eastern Cooperative Oncologic Group; ER: Estrogen receptor; MCT: Metronomic chemotherapy; PgR: Progesterone receptor.

pre-established number of 25 patients. Baseline characteristics of patients and tumors are depicted in Table 1. The median age was 61 years (range: 38–78 years). All patients were heavily pretreated; seven out of 15 patients and eight out of 15 received three and four prior chemotherapy regimens, respectively. The most frequent metastases locations were liver and bone.

Toxicity & adverse events

The hematologic adverse events associated with the therapy were: anemia grade 2 (n = four out of 15); leukopenia grade 1 (n = two out of 15) and grade 2 (n = two out of 15); neutropenia grade 1 (n = one out of 15) and grade 2 (n = two out of 15); and thrombocytopenia grade 1 (n = one out of 15). Those events were easily overcome with transitory reduction of Cy doses [101]. Four patients out of 15 developed grade 1 dyspepsia related to the treatment, a toxicity that did not affect compliance and was reversed easily with the administration of omeprazole 20 mg *per orem* daily. All adverse events, related or not related to the therapy, are summarized in Table 2.

There was no evidence of hepatic, renal or cardiac toxicities associated with the therapy. Minimal changes in liver enzymes (lactate dehydrogenase and alkaline phosphatase) were probably due to the underlying disease or related to disease progression. CA15-3 concentrations increased at the time of disease progression and those values, along with the CT scans, were useful in identifying patients who had progressed [102].

Therapeutic response

A PR was observed in one patient (RR: 6.7%) that lasted 6 weeks. pSD was observed in six out of 15 patients (40%). The OCB obtained was 46.7% (PR: one out of 15 and pSD: six out of 15). Median TTP was 14 weeks and TTP among patients with pSD was 37.5 weeks (range: 26.43–81.57). PFS at 24 weeks was 40% (Figure 1A). The OS rate 1 year after enrollment in the protocol was 46.7% (Figure 1B) and the median OS was 45 weeks (range: 5–153).

In the multivariate analysis, the chance of pSD or PR was not significantly associated with negative hormone receptor status (odds ratio: 0.177; p = 0.263) or baseline high ECOG status (odds ratio: 0.564; p = 0.736).

Biomarkers

The percentages of CECs and CEPs did not show significant modifications during the treatment. The same result was obtained when patients were classified as nonprogressive (Figure 2A & C) and progressive (Figure 2B & D). Baseline values of CECs and CEPs did not correlate with TTP (r^2: 0.02 and p = 0.29; r^2: 0.019 and p = 0.31, respectively).

Serum concentration of VEGF decreased as a function of time (p = 0.004) (Figure 3A). On the other hand, TSP-1 levels did not show significant modifications (Figure 3B), even when patients were classified as progressive and nonprogressive. A negative and significant correlation between baseline values of VEGF and TTP was found (p = 0.358 and r^2: 0.2654), while that was not the case for TSP-1.

Life quality

FACT-B questionnaire

During follow-up, when comparing the FACT-B items during response from baseline to middle of treatment no significant differences were observed. When questionnaires corresponding to the end of treatment were included in the statistical analysis, the item additional concerns showed a significant increase (p = 0.008), while the increase for functional well being was marginally significant (p = 0.055) [103].

Pain questionnaire

Considering the percentage of patients with pain and its intensity at baseline (80%; median [range]: 4 [0–8]), middle of treatment (73.3%; 3 [0–6]) and end of treatment (92.3%; 3 [0–7]), no significant differences were observed among the groups. Therefore, treatment neither led to pain relief nor pain increase. Interestingly, one

Table 2. Adverse events during treatment.

Adverse event	Grade 0; n (%)	Grade 1; n (%)	Grade 2; n (%)	Grade 3; n (%)
Hematologic				
Anemia	8 (53)	1 (7)	4 (27)	2
Leukopenia	10 (67)	2† (13)	2† (13)	1 (7)
Neutropenia	11 (73)	1† (7)	2† (13)	1 (7)
Thrombocytopenia	14 (93)	1† (7)	–	-
Nonhematologic				
Dyspepsia	11 (73)	4† (27)	–	–
Diarrhea	11 (73)	2 (13)	2 (13)	–
Nausea	10 (67)	5 (33)	–	–
Vomiting	14 (93)	1 (7)	–	–
Anorexia	10 (67)	4 (27)	1 (7)	–
Weight loss	14 (93)	1 (7)	–	–
Asthenia	12 (80)	3 (20)	–	–
Urticaria	14 (93)	1 (7)	–	–
Dyspnea	10 (67)	4 (27)	1 (7)	–
Hypertension	14 (93)	1 (7)	–	–
Edema	13 (87)	1 (7)	1 (7)	–
Cough	10 (67)	4 (27)	–	–
Fever	10 (67)	4 (27)	–	–
Ascites	12 (80)	1 (7)	1 (7)	1 (7)
Pleural effusion	13 (87)	1 (7)	1 (7)	–
Pruritus	14 (93)	1 (7)	–	–
Abdominal pain	14 (93)	1 (7)	–	–
Muscular pain	13 (87)	2 (13)	–	–
Arthralgia–bone pain	2 (13)	8 (53)	4 (27)	1 (7)
Alopecia	13 (87)	2 (13)	–	–
Lymphatic edema	13 (87)	1 (7)	1 (7)	–
Depression	9 (60)	6 (40)	–	–

There was no evidence of grade 4 toxicity.
†Associated with treatment.

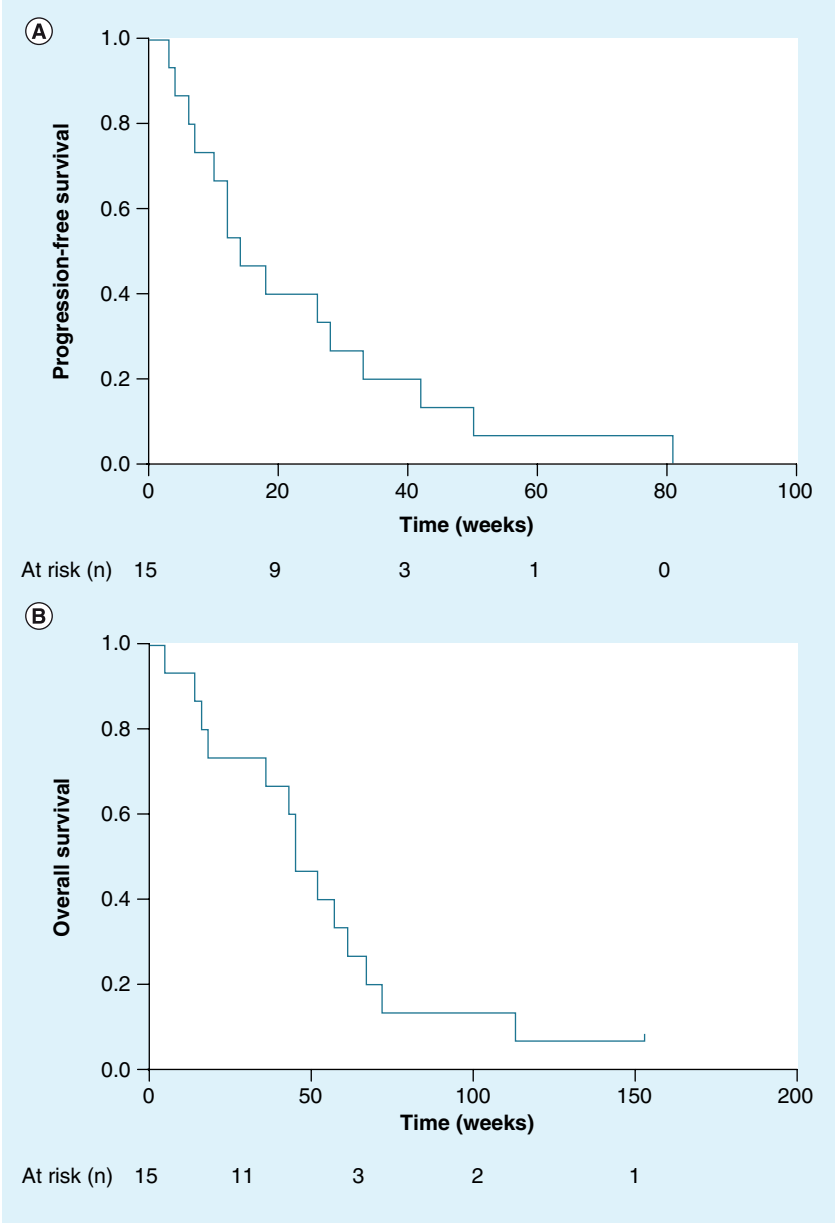

Figure 1. Progression-free and overall survival during treatment.
(A) Progression-free survival and **(B)** overall survival.

patient decreased the opioid dose during 5 weeks and another improved her walking [104].

ECOG evaluation

In assessing the performance status according to the ECOG scale, the value corresponding to the TTP was not included. Four patients out of 15 (26.7%) were not evaluated since their permanence in the treatment was ≤8 weeks. The analysis showed no changes in the score in one out of 11 (9%) patients, a worsened score in four of 11 (36.4%) patients, and a transitory and a permanent improved score in two (18.2%) and four out of 11 (36.4%) patients, respectively [105].

Discussion

Effective, noninvasive and low-toxicity treatments for breast cancer represent a challenge for medical oncologists. For decades the pharmaceutical industry has been focused on developing antineoplastic agents with a high-cytotoxic effect on tumor cells. This effect is related to agent doses and is the main cause of anti-tumor efficacy; however, it is also accompanied by toxicity and deterioration of patients' quality of life.

Angiogenesis represents a key process in carcinogenesis. Antiangiogenic drugs interfere in many common pathways shared in all neoplastic cells in a nonspecific fashion [25]. The mechanism of action of MCT proposed by Folkman and Kerbel points to the tumor endothelial cells that, indirectly, destroy naive and resistant cancer cells by induction of hypoxia and nutrient deprivation [3,4]. These mechanisms had been validated in preclinical and clinical models. However, additional mechanisms of action may be involved such as induction of tumor dormancy and immunomodulation [10,12].

Based on our preclinical studies, we designed this trial to evaluate the safety and efficacy of MCT with Cy plus Cel in advanced breast cancer, refractory to standard chemotherapy, along with the identification of possible biomarkers to predict the response to therapy [18].

The OCB obtained was 46.7% (PR: one out of 15 and pSD: six out of 15). This result is very encouraging considering the stage of the disease and the low possibility of obtaining a long-term clinical benefit after three or four lines of chemotherapy.

Our findings agree with others who employed comparable therapeutic approaches. Colleoni *et al.* treated metastatic breast cancer patients with metronomic Cy plus methotrexate (Mtx) and obtained an OCB of 31.7% (CR + PR + stable disease: ≥ 24 weeks); despite this only 14% of patients were heavily pretreated and 80% of them had progressive disease. Moreover, we found similar low toxicities and decreased serum VEGF levels [26]. Later on, the same authors, enrolling a higher number of patients, 70% of which had progressive disease, obtained an OCB of 41.5% in patients treated with Cy and Mtx with or without thalidomide [27]. Other therapeutic approaches utilized metronomic Cy as a single agent, which provided stable disease in heavily pretreated patients [28], or in combination with other agents such as Cy plus Mtx and trastuzumab in Her2/neu$^+$ patients [29]. The potential uses of Cy-based MCT were recently discussed [30]. In addition, it was demonstrated

that MCT with capecitabine displayed good therapeutic activity in advanced breast cancer [31] and that MCT in combination with bevacizumab and erlotinib was effective in HER2$^{(-)}$ advanced breast cancer [32]. Also, in patients with colorectal cancer at high risk of recurrence, adjuvant post-operative MCT using irinotecan plus uracil and tegafur improved OS rates [33]. Moreover, patients with advanced hormone-refractory prostate cancer treated with a single standard dose of Cy followed by MCT with Cel and dexamethasone showed biological activity and a low-toxicity profile [34].

Interestingly, the therapeutic results obtained so far in metronomic trials for metastatic breast cancer patients are similar to those achieved with standard chemotherapy, but they have the advantage of avoiding toxicity, the main drawback of maximun tolerated dose regimens [35,36].

On the other hand, in a large Phase II trial including heavily pretreated patients with different types of cancer (breast, gastrointestinal, melanoma, ovarian, prostate, renal) and treated with daily Cy and Cel and weekly Mtx, Khan *et al.* found low toxicity, but also nonobjective response and a short duration of stable disease (34.3% ≥12 weeks) [37]. The inclusion of patients with other types of cancer (78%) in the study could account for the different efficacy achieved.

We documented PR in 6% and pSD in 40% of the patients with a median TTP of 37.5 weeks in this group and a PFS at 24 weeks of 40%. These results get close to those found by Orlando *et al.* with a PR in 18% and pSD in 46% of patients, and a median TTP of 24 weeks [29]. This is higher than those found by Salem *et al.*, with pSD in 14.3% patients and median response duration of 4 ± 0.84 months [38], and Wong *et al.* who obtained 2% CR, 15% PR and 7% pSD, with a median TTP of 10 weeks [39].

We found very low toxicity, with myelosuppression the most common event associated to Cy treatment. Interestingly, this was easily reverted by temporary dose reduction. Gastrointestinal toxicity was also minimal. Compared with other standard schemes of treatment, the side effects derived from the present treatment were minimal [40].

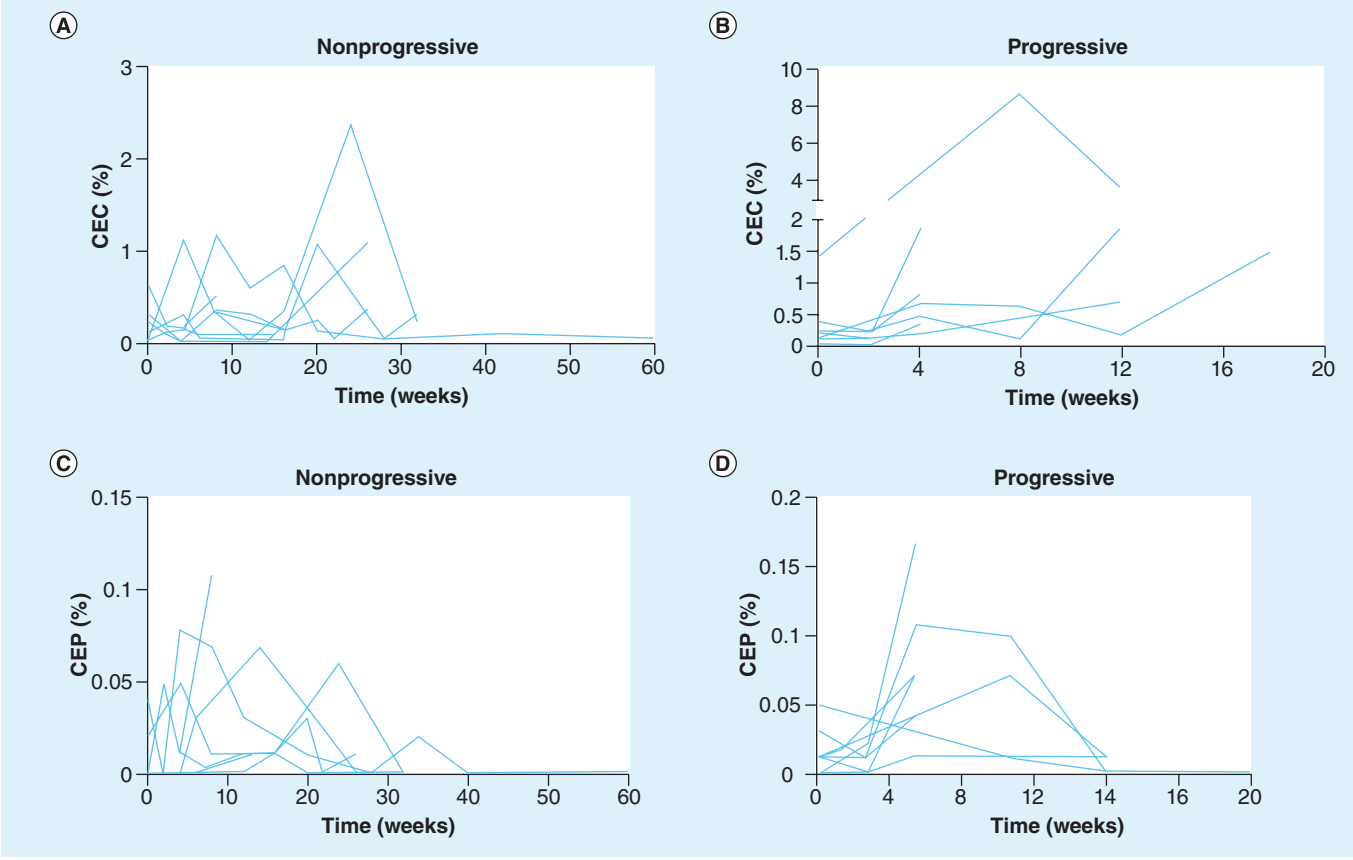

Figure 2. Circulating endothelial cells and circulating endothelial progenitor cells during treatment. Individual variation of percentage of **(A & B)** CECs and **(C & D)** CEPs in nonprogressive (n = 7) and progressive (n = 8) patients, respectively.
CEC: Circulating endothelial cell; CEP: Circulating endothelial progenitor cell.

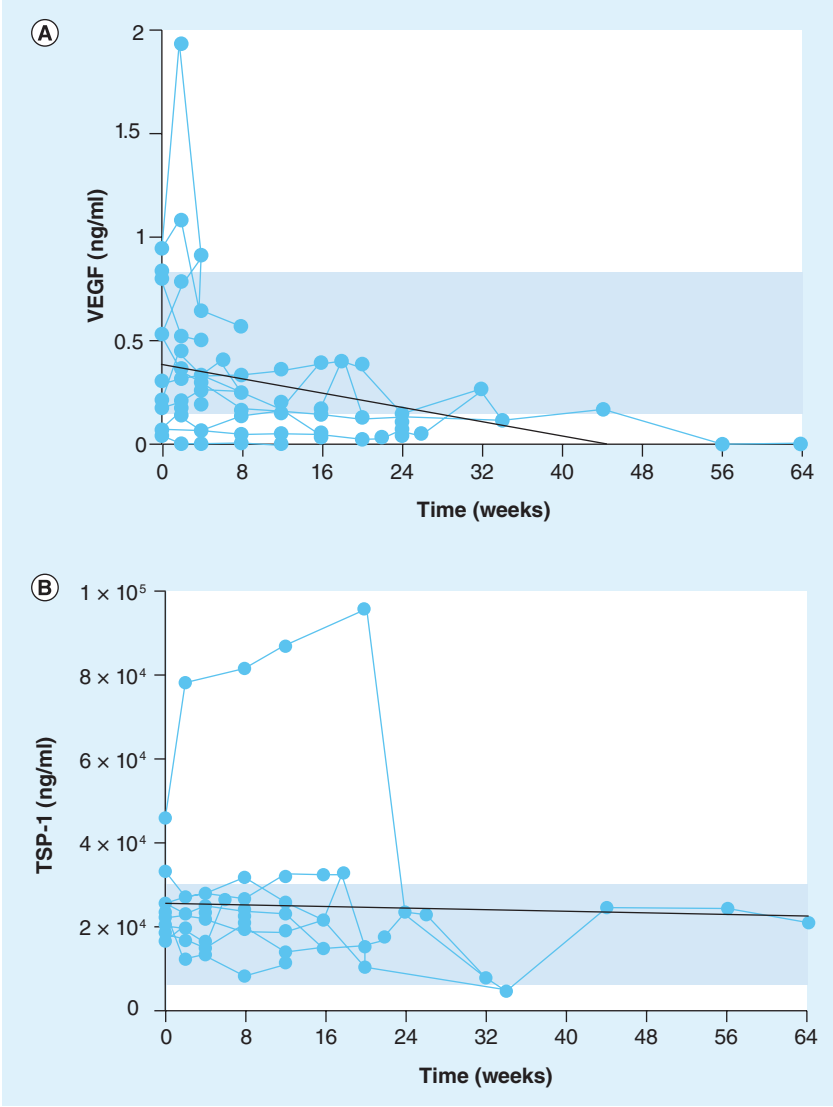

Figure 3. Serum levels of VEGF and thrombospondin-1. Individual variations of **(A)** VEGF and **(B)** TSP-1 serum concentrations. The gray zones indicate the range of normal values. Linear regression analysis: **(A)** r^2: 0.113, p = 0.0042; and **(B)** r^2: 0.002, p = 0.71.
TSP-1: Thrombospondin-1.

Several authors have demonstrated, in preclinical models, the therapeutic effect of metronomic administration of Cy in combination with various antiangiogenic agents, such as angiogenesis inhibitor TNP-470 [3], imatinib [41], the angiogenic peptide ABT-510 [42], tirapazamine [43], cetuximab, anti-EGF receptor [44], 5-fluorouracil prodrug UFT [45], the angiogenesis inhibitor axitinib [46] and others, in the treatment of different types of tumors, including lung cancer and leukemia [3], pancreatic carcinoma [41], prostate [42], colon [43] and breast cancer [45].

One of the most important angiogenic molecules is VEGF, which stimulates adhesion, proliferation and survival of normal endothelial cells. While its expression is strictly regulated in normal tissue, the overexpression of VEGF has been observed, in several tumor models, to be associated with abnormal blood vessels [7]. On the other hand, antiangiogenic factors, such as TSP-1, inhibiting proliferation, growth, motility and adhesion of tumor endothelial cells may be involved in the regulation of tumor angiogenesis [47]. In addition, the recruitment of hematopoietic cells and CEPs derived from bone marrow are necessary for tumor angiogenesis [48]. In addition the CEPs, which can be mobilized from the bone marrow by growth factors, such as VEGF, cytokines or ischemia [49], would be a MCT cell target [50].

In order to find possible markers of response to therapy, we evaluated serum levels of VEGF and TSP-1, and percentages of CECs and circulating CEPs. Serum VEGF showed a significant decrease over time, supporting the antiangiogenic effect of the treatment. Interestingly, lower VEGF baseline values were significantly correlated with longer TTPs. However, the limitations in the number of patients included so far preclude assuring its usefulness as a predictor of response. Different results were obtained in a Phase II study conducted with bevacizumab and a metronomic oral dose of Cy in patients with recurrent ovarian tumors, in which no correlation was found between VEGF levels and response to treatment [51]. On the other hand, MCT treatment with daily Cy, dalteparin and prednisone, and twice-weekly doses of Mtx in patients with metastatic breast cancer showed clinical activity, a nonsignificant decrease of VEGF, and a significant increase in receptors sVEGFR-1 and -2 after 2 weeks of therapy [39], although a combination of Cy, Cel and Mtx showed negligible activity in advanced cancers and the analysis of several biomarkers (including VEGF) indicated minimal effects on endothelium [37].

TSP-1 is a potent and highly specific inhibitor of angiogenesis, and is considered a secondary mediator of the antiangiogenic effect of some MCT regimens [52]. However, our results showed no significant modifications in TSP-1 serum concentration. Moreover, those values did not correlate with disease progression or treatment response. Accordingly, several studies also failed to find such a correlation [34,53,54].

The proportion of CEPs and CECs varied during treatment without showing a clear tendency. On the other hand, a study in ovarian cancer patients showed that high circulating CEPs correlated with poor OS [55]. Other studies

in Hodgkin lymphoma [20] and breast cancer [19] patients showed a decrease in CEPs and/or CECs when treated with Cy plus Cel, or Cy plus capecitabine and bevacizumab, respectively.

One common outcome when employing maximum tolerated dose regimens is deterioration of patients' quality of life. On the contrary, we found that quality of life showed no changes during the response period. This is an interesting finding, considering the type of patients we are dealing with. When the questionnaire corresponding to the end of treatment was also included for statistical analysis, the only item that showed a significant increase was additional concerns. The increase in additional concerns could be due to fear, frustration or the patients' knowledge of a lack of additional curative treatments. Moreover, assessment of functional status using the ECOG scale showed that 70% of patients' conditions improved or did not change. Such a result may be mainly attributed to stable disease, noninvasive drug administration, low toxicity of the treatment, the anti-inflammatory effect of Cel and, also, to a subjective factor from the patient. The control of pain plays an important role in cancer treatment and directly influences the quality of life. The intensity of pain remained stable in our patients who demonstrated a nonsignificant decrease in its median intensity. The absence of a significant improvement in pain relief could be attributed to the advanced disease present in the patients. Gebbia *et al.* found that pain intensity decreased or remained stable in 63% of patients with metastatic prostate adenocarcinoma treated with metronomic Cy and Mtx [56]. Importantly, André *et al.* found a decrease in pain and antialgic drug use in 68.75% of the patients after initiation of a four drug regimen metronomic treatment for different kinds of tumors in children with refractory disease and no further effective treatment available [57].

Interestingly, some repositioned agents, such as propanolol and metformin, have broadened the range of drugs with potential utility in MCT; both drugs are endowed with interesting properties such as therapeutic efficacy, low toxicity, oral administration and, last but not least, low cost. Metronomic administration of propranolol combined with other chemotherapy drugs potentiated the anti-tumor effect of chemotherapy drugs and may improve relapse-free and OS in breast cancer patients [58]. In addition, the results obtained with metformin in the neoadjuvant setting by Niraula *et al.* in early breast cancer patients [59] are encouraging. Likewise, we have obtained a significant survival increase in mice bearing a mammary adenocarcinoma treated with a metronomic combination of Cy and metformin [Basualdo, Jesús O, Unpublished Data].

In conclusion, MCT combining Cy and Cel for treating advanced breast cancer showed very low toxicity and therapeutic response in almost half of the patients. The decrease of serum VEGF confirmed the antiangiogenic nature of the drug combination and therapeutic schedule and its baseline value showed its potentiality as a predictor of TTP. There was no deterioration in the quality of life in a high proportion of patients, a result that represents one of the most important rewards, considering their stage of disease progression at the beginning of the therapy.

Future perspective

The promising results obtained in the first stage of the trial warrants not only its progression to the second stage, but also the design of new protocols targeting patients in earlier stages of the disease on its completion.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors declare that the protocols herein described comply with the current laws of Argentina. The protocol was authorized by the School of Medicine Bioethics Committee and by ANMAT (Argentine Regulatory Agency). The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Therapeutic response

- The overall clinical benefit obtained was 46.7% (partial response: one out of 15 + prolonged stable disease: six out of 15). The median time to progression in patients with prolonged stable disease was 37.5 weeks. Progression-free survival at 24 weeks was 40%. The overall survival rate 1 year after enrollment in the protocol was 46.7% and the median overall survival was 45 weeks (range: 5–153).

Biomarkers

- Serum concentration of VEGF decreased significantly as a function of time and VEGF baseline values were negatively and significantly correlated with time to progression.

Toxicity & life quality

- The adverse events related to the therapy were few and mild (grade 1 or grade 2), mainly hematologic and gastrointestinal.
- When comparing the Functional Assessment of Cancer Therapy Breast items during response, no significant differences were observed.
- The performance status measured by the Eastern Cooperative Oncologic Group scale showed no modification or improvement in seven out of 11 evaluable patients (63.6%) of the responder patients.

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