Clinical and Pharmacodynamic Evaluation of Metronomic Cyclophosphamide, Celecoxib, and Dexamethasone in Advanced Hormone-refractory Prostate Cancer

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Abstract

Purpose: The aims of the present study were to evaluate the clinical activity and the pharmacodynamic profile of the novel schedule of a single i.v. standard dose of cyclophosphamide (CTX) immediately followed by an oral metronomic CTX regimen with celecoxib (CXB) and dexamethasone (DEX) in advanced hormone-refractory prostate cancer patients.

Experimental Design: Twenty-eight patients (68% docetaxel-resistant) received 500 mg/m² CTX i.v. bolus on day 1 and, from day 2, 50 mg/day CTX p.o. plus 200 mg/twice a day CXB p.o. and 1 mg/day DEX p.o. until disease progression. Plasma vascular endothelial growth factor (VEGF) and thrombospondin-1 were detected by ELISA, and real-time reverse transcription-PCR of VEGF and thrombospondin-1 gene expression on peripheral blood mononuclear cell and of VE-cadherin (VE-C) in blood samples was done.

Results: A confirmed prostate-specific antigen decrease of ≥50% from baseline was observed in 9 of 28 patients (32%). Median progression-free survival and overall survival were 3 months (95% confidence interval, 2.2-4.2 months) and 21 months (95% confidence interval, 12.4-29.4 months), respectively. Toxicity was mild and no grade 3 to 4 toxicities occurred. A significant relationship was found between plasma VEGF and prostate-specific antigen values \( (r = 0.4223; P < 0.001) \). VEGF levels significantly increased in nonresponders, whereas the responder patients maintained significantly lower levels of VE-C gene expression after the beginning of the treatment if compared with nonresponder ones.

Conclusion: Metronomic CTX plus CXB and DEX showed favorable toxicity and activity profile in patients. VE-C gene expression and VEGF levels represent potentially useful pharmacodynamic markers for the clinical response.

Metronomic oral cyclophosphamide (CTX) alone—the chronic administration of CTX at low doses with no prolonged drug-free breaks—and in association with antiangiogenic drugs is a promising clinical approach to metastatic breast and ovarian cancers (1, 2). The antitumor and antiangiogenic activity of metronomic CTX has been shown in various experimental models of human prostate cancer (3). Phase II clinical studies have obtained interesting results using metronomic oral CTX alone (50 mg/m²/day; ref. 4) or in combination with dexamethasone (DEX; 50 mg/day CTX plus 1 mg/day DEX; ref. 5) in hormone-refractory prostate cancer (HRPC) patients. Metronomic CTX was safe and well-tolerated, showing a prostate-specific antigen (PSA) of ≥50% decline in 69% of patients with a response duration of 8 months (5). The administration of metronomic CTX with drugs targeting angiogenesis has shown a high preclinical activity (6, 7). Celecoxib (CXB), a cyclooxygenase-2 inhibitor, has shown both antiangiogenic properties (8) and antitumor activity in a transgenic mouse model of prostate adenocarcinoma (9) with a marked decrease in vascular endothelial growth factor (VEGF) expression (10). Moreover, CXB phase II clinical trials in patients with a PSA recurrence (after radical prostatectomy and/or radiotherapy for their localized disease) have shown an interesting activity (in terms of slowing PSA kinetics) without relevant toxicities.

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Translational Relevance

Metronomic chemotherapy is a promising approach to the therapy of solid tumors because it presents some advantages compared with standard chemotherapy: reduced toxicity, activity in chemotherapy-resistant cancer, and lower costs. The antiangiogenic activity of metronomic chemotherapy is being exploited to target a key process to tumor progression, which is the growth of new blood vessels. However, when addressing the issue of metronomic chemotherapy, it is instrumental to establish a correlation between tumor response and biological endpoints, i.e., markers of angiogenesis such as vascular endothelial growth factor, thrombospondin-1, and VE-cadherin to confirm the metronomic effect of treatments delivered to patients. In this study, the metronomic schedule of cyclophosphamide in combination with celecoxib and dexamethasone was delivered to advanced hormone-refractory prostate cancer patients and clinical outcome was related to markers of angiogenesis to validate the characteristics of this treatment and assess its clinical profile.

Patients and Methods

Study design and patient selection. The present multicenter, prospective, nonrandomized phase II clinical study was approved by the local ethics committee and registered in the European Clinical Trial Database EudraCT (registration number 2005-005967-27). Patients were informed of the investigational nature of the study and provided their written informed consent for the study treatment and related procedures. Eligibility criteria at baseline included age of ≥18 y, histologic diagnosis of prostate adenocarcinoma, hormone-refractory metastatic disease, failure of one or more previous chemotherapeutic lines of treatment for metastatic disease. Chemotherapy-naïve patients were also included in this study if they refused standard regimens due to toxicity concerns. Other inclusion criteria were as follows: measurable disease progression and/or increasing PSA serum level due to toxicity concerns. Other inclusion criteria were as follows: measurable disease progression and/or increasing PSA serum level due to toxicity concerns.

Table 1. Patient characteristics (n = 28)

<table>
<thead>
<tr>
<th>Age</th>
<th>Median (Range)</th>
<th>No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Cooperative Oncology Group performance status</td>
<td>0 12/43</td>
<td>1 16/57</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>28/100</td>
<td></td>
</tr>
<tr>
<td>Sites of metastatic disease</td>
<td>Bone 23/82</td>
<td>Nodes 10/36</td>
</tr>
<tr>
<td>No. of involved organs</td>
<td>1/2 26/93</td>
<td></td>
</tr>
<tr>
<td>Measurable disease (RECIST criteria)</td>
<td>Yes 5/18</td>
<td>No 23/72</td>
</tr>
<tr>
<td>Prior hormonal manipulations</td>
<td>1 2/7</td>
<td>2 5/18</td>
</tr>
<tr>
<td>Prior radiotherapy</td>
<td>6/21</td>
<td></td>
</tr>
<tr>
<td>Prior prostatectomy</td>
<td>8/28</td>
<td></td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td>19/68</td>
<td></td>
</tr>
<tr>
<td>1 line 4/14</td>
<td>2 lines 5/18</td>
<td>&gt;2 lines 10/36</td>
</tr>
<tr>
<td>Docetaxel-based chemotherapy</td>
<td>19/68</td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone plus prednisone</td>
<td>13/46</td>
<td></td>
</tr>
<tr>
<td>Serum PSA</td>
<td>Median (ng/mL) 63.2</td>
<td>Range 10-5,000</td>
</tr>
<tr>
<td>Gleason score</td>
<td>&lt;7 3/11</td>
<td>≥7 18/64</td>
</tr>
<tr>
<td>Not available 7/25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain VAS scale ≥1 10/35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSP-1 pretreatment levels, ng/mL mean ± SD 287 ± 183</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF pretreatment levels, pg/mL mean ± SD 105 ± 101</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(11–13). Glucocorticoids are used for the treatment of HRPC as a single agent or in combination with chemotherapy (14). Recently, Yano and colleagues (15) have shown that DEX could exert an antiangiogenic and antitumor activity via down-regulation of VEGF and interleukin-8 expression in an in vivo model of HRPC. Low doses (0.5 and 2 mg/day) of DEX have been also evaluated in HRPC, reaching a PSA of ≥50% decline in ~60% of patients (16, 17).

The aims of the present study were to evaluate in advanced HRPC patients the clinical activity and the pharmacodynamic profile of a single i.v. standard dose of CTX immediately followed by an oral metronomic CTX regimen with CXB and DEX. As potential molecular markers of the antiangiogenic activity of the combination, we explored the gene expression and protein variations of thrombospondin-1 (TSP-1), VEGF, and VE-cadherin (VE-C) induced by treatment.

haemoglobin level, ≥10 g/dL; platelets, ≥100,000/mL; adequate liver function (total serum bilirubin level, <1.5 mg/dL; aspartate aminotransferase and alanine aminotransferase, <3 upper normal limit); adequate renal function (serum creatinine level, <1.5 mg/dL), and life expectancy of ≥3 mo.

Antiandrogen therapy (flutamide and bicalutamide) was discontinued at least 6 wk before enrollment, at which point evidence for PSA progression was required. The use of low-dose megestrol acetate for amelioration of symptoms was allowed. LHRH analogues, corticosteroids, and the zoledronic acid were allowed if started at least 4 wk before the study and associated by a rising PSA.

Exclusion criteria at baseline were as follows: uncontrolled metabolic diseases, cardiovascular disease (uncontrolled hypertension and arrhythmia, myocardial infarction within 2 y before enrollment, unstable angina, New York Heart Association, grade II or greater congestive heart failure), active infections, high risk of thromboembolic events without prophylactic treatments, untreated haemorrhagic gastric disease, or presence of brain metastases.

Treatment. All eligible patients received on day 1 a single administration of CTX 500 mg/m2 as i.v. bolus and, from day 2, 50 mg CTX p.o. once daily plus 200 mg CXB p.o. twice a day and 1 mg...
DEX p.o. daily. From day 2, the treatment was continued without interruption until unacceptable toxicities, disease progression, deterioration of performance status, or patient's refusal. CTX was withheld until recovery in case of an absolute neutrophil count of <1,000/μL and/or platelets count of <75,000/μL and/or if a National Cancer Institute-Common Toxicity Criteria v.2.0 grade 2 or greater mucositis, gastrointestinal, or other toxicities occurred. No dose reduction for toxicities was applied.

Clinical evaluation. Pretreatment evaluations included the following: medical history, a physical exam with an assessment of weight, vital signs, and Eastern Cooperative Oncology Group performance status; electrocardiogram plus cardiovascular examinations, complete blood count, complete serum biochemistry (creatinine, glyceria, sodium, potassium, calcium, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, \( \gamma \)-GT, ALP, total bilirubin, PT, aPTT, fibrinogen, and D-dimer), PSA serum levels, pain evaluation according to the visual analogical scale (VAS), and measurable disease evaluation, if applicable. During treatment, each patient did the following every 2 wk: physical examination, toxicity (NCI-CTC) record, blood count, serum creatinine; every 4 wk: blood count, partial serum biochemistry (creatinine, total bilirubin, calcium, PT, aPTT, fibrinogen, and D-dimer); every 8 wk: electrocardiogram, physical and cardiovascular examination, toxicity (NCI-CTC) record, blood count, complete serum biochemistry, and measurable disease evaluation. PSA serum levels were measured every 2 wk.

Table 2. Maximum toxicity per patient (n = 28)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>G1</th>
<th>G2</th>
<th>G3-G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>4 (14)</td>
<td>1 (3.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7 (25)</td>
<td>1 (3.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Anemia</td>
<td>18 (64)</td>
<td>3 (11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>10 (36)</td>
<td>4 (14)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1 (3.6)</td>
<td>1 (3.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cardiovascular Events</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Fig. 1. A, waterfall plot showing the maximal (at 12 wk or at any time point) PSA after therapy change from baseline. B, actuarial progression-free survival (PFS) and overall survival (OS) curves calculated by the Kaplan-Meier method from the first day of the combined metronomic CTX, CXB, and DEX schedule.
titation of gene expression was done using the malcycling conditions and optimization of primer concentrations were purchased from Applied Biosystems (Assay ID Hs00170236_m1, and the quan-
malcycling conditions and optimization of primer concentrations were purchased from Applied Biosystems (Assay ID Hs00170236_m1, and the quan-
was normalized to glyceraldehyde-3-phosphate dehydrogenase, and the quan-
alcycling conditions and optimization of primer concentrations were purchased from Applied Biosystems (Assay ID Hs00170236_m1, and the quan-
was normalized to glyceraldehyde-3-phosphate dehydrogenase, and the quan-
and TSP-1 levels using commercially available ELISA and EIA kits. Each sample was assayed for human VEGF and TSP-1 concentrations by the ELISA kit VEGF Quantikine (R&D Systems) and by the ChemiKine Human TSP-1 EIA kit (Chemicon), respectively. The absorbance was determined using the microplate reader Multiskan Spectrum (Thermo Labsystems) set to 450 nm (with a wavelength correction set to 540 nm) for the VEGF kit and 490 nm for TSP-1 kit. The data are presented as the percentage of the VEGF and TSP-1 plasma levels at day 0.

**Endpoints and response criteria.** The primary end point of the study was PSA-based outcome defined by a decrease of ≥50% from baseline, maintained for at least 4 wk, in accordance with the consensus guidelines of the Prostate Cancer Clinical Trials Working Group 1 (18). PSA progression was defined as a 50% or greater increase and an absolute increase of 5 ng/mL or more from the nadir, which is confirmed by a second value obtained 2 or more wk later. Where no decline from baseline is documented, PSA progression was defined as a 25% confirmed increase from the baseline value along with an increase in absolute value of 5 ng/mL (18). Secondary end points were as follows: objective response rate according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria, toxicity (NCI-CTC), duration of PSA response, time to PSA progression, pain control (VAS), overall survival, ability to modulate plasma TSP-1 and VEGF levels, gene expression of TSP-1, VEGF and VE-C in peripheral mononuclear blood cells, and whole blood during treatment, respectively.

**Statistical analyses.** According to Simon’s minimax two stage design with \( P_0 = 20\% \), \( P_1 = 40\% \), \( \alpha = 0.10 \), and \( \beta = 0.20 \), the enrollment of 14 patients was required in the first step of the study. If at least 2 objective and/or PSA responses were observed, a total of at least 24 assessable patients were enrolled. Study treatment was considered interesting if at least 7 of 24 patients responded. Time to progression and overall survival were calculated from the date of first chemotherapy administration to the date of progression or death/loss to follow-up, respectively. Time to progression and overall survival were analyzed according to the Kaplan-Maier method. Response duration was calculated from the time of first objective response to the time of disease progression. An intent to treat analysis was done.

Statistical analysis by ANOVA, followed by the Student-Newman-Keuls test, was used to assess the statistical differences of pharmacodynamic data. \( P \)-values of ≤0.05 were considered significant. Correlations between TSP-1, VEGF, and PSA parameters were investigated by linear regression analysis. Statistical analyses were done using the GraphPad Prism software package version 4.0 (GraphPad Software, Inc.).

**Results**

**Characteristics of the patients and treatment.** Twenty-eight patients from two institutions were enrolled into the study and were assessable. Baseline patient characteristics are listed in Table 1. Furthermore, 15 patients (53.6%) received two or more chemotherapeutic lines that included the following: mitoxantrone (13 patients, 46%), estramustine phosphate (17 patients, 61%), vinorelbine (3 patients, 11%), etoposide (2 patients, 7%), and methotrexate (1 patient, 3%). Zoledronic acid was administered to 19 patients (68%) with bone metastases. The median duration of metronomic chemotherapy was 5.4 months (range, 1.4-21.5 months).

**Toxicity.** The initial standard CTX single i.v. dose and the following metronomic CTX plus CBX and DEX were very well-tolerated. Indeed, no NCI-CTC grade 3 to 4 toxicities were reported in the 28 assessable patients. One patient (3.6%) developed NCI-CTC grade 2 neutropenia that recovered after 2 weeks of rest from treatment. Only one patient (3.6%) experienced grade 2 thrombocytopenia that required CTX discontinuation. In such cases, the adverse event occurred after ~16 months of...
treatment. Neither major cardiovascular events nor toxicity-related deaths were observed (Table 2).

Activity. According to the recommendations of the Prostate Cancer Clinical Trials Working Group 1 (18), a confirmed PSA decrease of ≥50% from baseline was observed in 9 (32%) of the 28 assessable patients. In Fig. 1A, a waterfall plot shows the percentage of change in PSA from baseline to 12 weeks and the maximal PSA decrease, as recommended by the most recent “Prostate Cancer Clinical Trials Working Group” guidelines (20).

Of note, among the nine responders, two subjects did not receive prior chemotherapy, whereas the remaining seven patients were previously given more than one line of chemotherapy (median, 2). Moreover, among the 19 nonresponders, 12 subjects were previously treated with more than one regimen (median, 2), whereas the other 7 patients were chemotherapy naïve. Based on these results, there was no statistical difference in the clinical activity of the metronomic CTX/DEX/CXB combination between patients previously untreated or treated with chemotherapy ($P = 0.6700$; Fisher’s exact test).

Moreover, five patients had a measurable disease according to RECIST criteria. No patient achieved a complete response, whereas one partial response (20%) and one disease stabilization (20%) were observed.

Median time to PSA response was 3 months (range, 1-11 months), and median duration of PSA response was 9.8 months (range, 4.9-19.3 months).

After a median follow-up of 20.5 months [95% confidence interval (CI), 17.9-23.0 months], median progression-free survival and median overall survival were 3 months (95% CI, 2.2-4.2 months) and 21 months (95% CI, 12.4-29.4 months), respectively (Fig. 1B). Ten of 28 patients (35%) had a basal VAS of ≥1 bone pain score, and among them, median baseline VAS was 3 (range, 1-8). Eight of 10 patients (80%) experienced pain relief with the best response after a median time of 42 days (range, 14-100 days) of treatment (median VAS value of 0; range, 0-1). Notably, 11 of 28 patients (39%) received the study treatment beyond PSA or disease progression in accordance to patient’s intent and due to the improvement of symptoms and quality of life. In such setting of patients, metronomic chemotherapy was administered for a median duration of 5 months (range, 2-12 months). Moreover, 9 patients (32%) received further chemotherapeutic lines after study treatment discontinuation.

Pharmacodynamic evaluations. To interpret the pharmacodynamic parameters in relation to the biochemical response, we defined as responders to the metronomic schedule 11 patients (39%) who had a decrease of PSA of ≥50% and a PSA stabilization of ≥6 months, a long-term biochemical control of the disease.

Relationships between PSA and VEGF or TSP-1 levels. A moderate but significant relationship was shown between plasma VEGF levels and PSA values ($r = 0.4223; P < 0.001$; Fig. 2A); indeed, for an increase of PSA, a moderately significant parallel increase of VEGF was recorded, suggesting that VEGF plasma levels may indicate the biochemical status of neoplastic disease. On the contrary, no statistically significant relationships were found between PSA concentration and TSP-1 plasma levels ($r = 0.0873; P = 0.266$; Fig. 2B).

Changes in VEGF and TSP-1 plasma levels. To compare the variations of plasma TSP-1 and VEGF before and during the drug treatments, graphs were drawn to show the concentrations as a percentage of the concentration at day 0 of individual patients before starting the metronomic CTX, CXB, and DEX schedule. Figure 3A shows that VEGF levels markedly increased in nonresponder patients and remained significantly higher than in responders for >80 days. In contrast, VEGF concentrations in responder patients constantly decreased to values corresponding to the half of the baseline (Fig. 3A). Figure 3B shows the results of the different profiles of TSP-1 plasma levels in the treated patients. Mean plasma TSP-1 levels, although with a high variability, increased during the treatment in both responder and nonresponders (Fig. 3B). However, no significant differences were noticed between the two groups. Interestingly, in responder patients, there was a simultaneous increase in TSP-1 levels and decrease of VEGF concentrations.
Modulation of VE-C, TSP-1, and VEGF gene expression. Figure 4A shows significant changes in VE-C gene expression. Interestingly, the responder patients maintained significantly lower levels of VE-C mRNA levels stayed around the baseline levels for 84 days (Fig. 4A). In contrast, the patients who did not respond to this therapy had a significant increase in VE-C gene expression already after 2 weeks, maintained high levels of VE-C for at least 2 months, and then started a slow decrease toward the baseline (Fig. 4A).

Figure 4B and C show VEGF and TSP-1, respectively, gene expression profiles in PBMC. VEGF expression profile in PBMC was similar to baseline, without any significant difference between responder and nonresponder patients (Fig. 4B). Similarly, no significant differences were found in TSP-1 gene expression between responders and nonresponders with the only exception at day 42 after the beginning of therapy (Fig. 4C).

Discussion

The present study describes, for the first time, the pharmacodynamic and clinical evaluation of a novel chemotherapy regimen consisting of an initial single i.v. CTX standard dose immediately followed by an oral metronomic CTX regimen with CXB and DEX in metastatic HRPC. Despite the recent demonstration of improved survival and quality of life with docetaxel-based chemotherapy in two large randomized phase III trials in patients with advanced metastatic HRPC, the majority of them will progress within 6 months and will require additional treatments (21, 22). To date, no standard second line chemotherapy is available in this setting of patients and new therapeutic strategies are warranted (23). Metronomic CTX chemotherapy has been shown, preclinically, to be an effective inhibitor of tumor angiogenesis (3) and of the mobilization of circulating endothelial cell progenitors (CEP; ref. 24). Moreover, low-dose CTX stimulates the production of TSP-1 (25), a potent endogenous inhibitor of angiogenesis, and induces a reduction of circulating regulatory T cells, leading to a restoration of peripheral T-cell proliferation and innate killing activities and favoring a better control of tumor progression (26). Clinically, such metronomic strategy presents some major advantages compared with standard chemotherapy, such as reduced toxicity, activity in chemotherapy-resistant cancer, and lower costs and the ease of using it in combination with other agents or antiangiogenic drugs (1, 27). Other compounds clearly showed antiangiogenic properties in preclinical prostate cancer models. Among them CXB (8), a selective cyclooxygenase-2 inhibitor, and DEX (15) present also an interesting activity in clinical trials. In our study, the combination of metronomic CTX plus CXB and DEX, in patients mainly resistant to docetaxel-chemotherapy (68% of the total enrolled), was feasible and showed a very favorable toxicity profile. Indeed, no grade 3 to 4 toxicity or cardiovascular events were observed even in the subgroup of patients continuously treated for >20 months.

Concerning the clinical activity, we observed a confirmed PSA of ≥50% decrease of 32% (95% CI, 16-48%) with a median duration of 9.8 months (range, 4.9-19.3 months). Interestingly, we obtained an overall PSA control rate (PSA response plus PSA stabilization, ≥6 months) of 39%. Di Lorenzo and colleagues (28) in a small phase I trial of metronomic CTX (50 mg p.o. daily) plus thalidomide (100-200 mg p.o. daily), conducted in 16 patients with advanced HRPC, observed a PSA response rate of 15% and a PSA stabilization of 8%. However, the 20% of patients suffered of grade 3 to 4 neutropenia and 10% of grade 3 to 4 anemia. The 9.8 months median response duration of our study resulted similar or higher than in the previously published experiences (7-8 months) both in early and advanced HRPC treated with oral CTX alone (4) or in combination with other drugs such as DEX (5), tegafur-uracil, and estramustine (29). Moreover, the interesting median overall survival of 21 months (95% CI, 12.4-29.4 months), observed in our study, could be partially explained by the fact that 39% of patients received metronomic therapeutically.
chemotherapy beyond biochemical or disease progression due to symptomatic and/or quality of life improvement and that 32% of patients received further chemotherapeutic lines. The data of the study by Glode et al. (5) showed a 69% of PSA response and 8 months of median response duration. Although the median response duration was similar to the one obtained in our prospective study (9.8 months), undoubtedly a great difference was found in terms of PSA response. These results may have been influenced by the retrospective nature of the Glode’s study and by the characteristics of patient population. Indeed, clinical trials should use a prospective design whenever possible to avoid the numerous biases related to the retrospective design (30). Moreover, in Glode’s clinical trial, the 85% of enrolled patients had a metastatic disease, whereas in the present study, they represented the 100%. Only the 38% of Glode’s patients resulted previously treated with chemotherapy (but no docetaxel was administered), whereas on the contrary, the 68% of docetaxel-resistant men were enrolled in the present trial. Moreover, Glode’s study presented bone and soft tissue metastasis in 55% and in 29% patients, respectively, whereas our patients resulted more severely involved, from a clinical perspective, because the 82% of them had bone metastasis and 47% had a soft tissue dissemination of the disease. Nelius and colleagues (31) in an unpublished small experience (17 patients) of metronomic CTX (50 mg p.o. daily) plus DEX (1 mg p.o. daily) in patients with taxane-resistant HRPC observed a PSA response rate of 23%. This study and the one by Di Lorenzo et al. (28) confirmed the differences with the Glode’s retrospective study in terms of PSA response and also support the rationale to combine metronomic CTX with CXB and the initial bolus of CTX. Indeed, the addition of CXB seems to improve (a) the percentage of PSA responses in our study if compared with the above mentioned prospective trials and (b) the median response duration of the published experiences (7-8 months). Moreover, preliminary results of the Prostacox phase II trial (32) shows a significant reduction of hematological toxicity of biweekly docetaxel administration combined with CXB, suggesting a possible role of CXB in the reduction of chemotherapy toxicity.

To date, several clinical trials have evaluated the activity of second line chemotherapy in HRPC patients progressed after docetaxel treatment. Mitoxantrone plus prednisone showed a ≥50% PSA decrease in only 6% to 20% of patients (33–35), and ixabepilone, a new epothilone B analogue, induced a PSA response of 17% with an overall survival of 9.8 months but with marked hematological toxicity such as grade 3 to 4 neutropenia in 54% of patients (34). Recently, the novel oral platinum compound satraplatin showed an interesting activity in a randomized phase III trial versus prednisone, with a PSA response rate of 25% (versus 12%; \( P = 0.00007 \)), a 31% risk reduction of progression-free survival events (hazard ratio, 0.69%; 95% CI, 0.60-0.80; \( P < 0.00001 \)), and a better pain control. However, 19.7% of patients required a satraplatin dose reduction and 39.4% at least 1 week dose delay. Moreover, grade 3 to 4 adverse events occurred with higher frequency in satraplatin-treated patients with severe fatigue and diarrhea and 15.9% of patients required RBC transfusions (versus 8% in prednisone-treated subjects; ref. 36).

The association of an initial single CTX high dose immediately followed by the metronomic regimen—to improve the chemotherapy responses without worsening the drug-related toxicity—was suggested by the results of Shaked et al. (37), who showed superior antitumor activity by combining intermittent high bolus doses plus continuous low doses of CTX in prostate PC-3 tumor model, and by the work of Pietras and Hanahan (38) who used a “chemo-switch” regimen consisting of an initial short course of MTD CTX, followed by long-term low dose oral CTX. Indeed, the CTX bolus of 500 mg/m² could reinforce the antiangiogenic and antitumor effects of metronomic CTX, CXB, and DEX trough different mechanisms: (a) a direct cytotoxic effect on cycling tumor endothelial cells as preclinically shown by Browder et al. (39) who found that a high bolus dose administered every 6 days induced potent antiangiogenic activity; (b) a direct cytotoxic effect on drug-sensitive tumor cells.

Using a quantitative reverse transcription-PCR approach for VE-C RNA evaluation, we have found the circulating levels of this endothelial-specific transcript to be significantly lower in responder patients compared with the nonresponder ones (a condition associated with a possible modulation of the angiogenic process). Rabascio and colleagues (40) observed a 2- to 300-fold reduction of VE-C RNA in apoptotic endothelial cells compared with viable cells \textit{in vitro} and that circulating VE-C levels were significantly reduced in patients achieving a partial remission compared with the ones in progression. In the same study, a significant relationship was found between increased number of circulating endothelial cells and bone marrow-derived CEPs and higher plasma VEGF levels and circulating VE-C RNA (40). Moreover, it has been shown that CTX and other chemotherapy drugs at standard doses can rapidly induce proangiogenic bone marrow-derived CEP mobilization and subsequent tumor homing (24, 41). On the other hand, Twardowski et al. (42) described that the combination of metronomic CTX at 50 mg daily with CXB at 400 mg twice daily did not significantly change the levels of circulating endothelial cell and CEP during therapy in cancer patients. These previously published data are in agreement with our findings and could explain, in part, our results. Indeed, although the above-mentioned advantages of initial high CTX dose, 500 mg/m² i.v. CTX probably determined an increase circulating endothelial cells and CEPs, as shown by the increase in VE-C RNA. However, in responder patients, this enhancement was significantly lower because of the immediate subsequent metronomic strategy blocking this process and maintaining a lower proangiogenic potential. After a prolonged period of time, the stimulus induced by the CTX bolus probably run out and the CEP mobilization was only sustained by the secreted VEGF of cancer cells, which was still significantly higher in nonresponders. The observed decrease of VEGF at day 80 (but still significantly higher than in responders) could have determined also the decrease of mobilized CEPs with the parallel reduction of VE-C expression.

A more sustained suppression of CEPs has been shown with the combination treatment (standard and metronomic dosing) when compared with CTX monotherapy regimens (37), suggesting a more efficacious anti-“vasculogenic” effect of the combined treatment. Moreover, the possible antiangiogenic mechanism of our combination therapy in responder patients is also suggested by the simultaneous significant decrease of plasma VEGF, as also described by Colleoni et al. (43), and the increase of the endogenous inhibitor of angiogenesis TSP-1, which would suggest a shift to an antiangiogenic state. Similar findings were previously described by our group in colorectal cancer patients treated with metronomic irinotecan (19).
Previously published data suggest that patients with metastatic prostate cancer have higher plasma VEGF levels than patients with localized disease or healthy controls (44). Some authors also found an inverse correlation between plasma VEGF levels and survival in patients with HRPC treated with suramin (an antiangiogenic/antitumor drug; ref. 45). Recently, Duque and colleagues (46) showed that patients with PSA levels of >20 ng/mL (with disseminated disease) had significantly higher VEGF values than patients with PSA levels of <20 ng/mL. Interestingly, our study described a moderate but significant correlation between VEGF plasma levels and serum PSA concentrations, suggesting that, at least in our study, VEGF plasma levels may indicate the biochemical status of neoplastic dissemination.

As also previously shown by Shafer et al. (47), we did not find any statistical correlation between PSA and TSP-1 in our patients. However, our data clearly suggested that the metronomic combination schedule determined an increase in plasma TSP-1 in all patients and some studies have found that TSP-1 levels inversely correlated with the amount of vasculature in prostatic tumors (48). Furthermore, some prostatic cancers became insensitive to the inhibitory effects of TSP-1, producing angiogenic factors such as VEGF (49). Indeed, our data seem to confirm these previous findings because nonresponder patients showed a simultaneous increase in both the proangiogenic and antiangiogenic endogenous factors, acting differently from the responder ones where, instead, an increase in TSP-1 and a decrease of VEGF levels was found.

In conclusion, for its favorable toxicity profile and its interesting activity, metronomic chemotherapy with CTX plus CXB and DEX could be proposed as a second line treatment in patients with HRPC or patients contraindicated to the treatment with standard cytotoxic chemotherapy. VE-C gene expression in peripheral blood and plasma VEGF and TSP-1 levels may represent interesting pharmacodynamic markers for the clinical response of HRPC patients treated with this novel schedule.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


