Exploratory predictive and prognostic factors in advanced breast cancer treated with metronomic chemotherapy

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The aim of the present study is to evaluate the clinical and biological factors (including markers of angiogenesis) as potential predictors of prognosis and benefit from metronomic therapy in patients with advanced breast cancer (ABC). Recent data suggest antiangiogenic activity of metronomic therapy. The study population included 62 patients with pretreated ABC who received cyclophosphamide and methotrexate orally. Tumour samples were analysed by immunohistochemistry for HER2, Ki-67, thymidine phosphorylase (TP), vascular endothelial growth factor and vascular endothelial growth factor receptor. The results from immunohistochemical analysis and clinico-pathological variables were studied to test their potential association with benefit from metronomic therapy. The median overall survival, progression-free survival and survival postprogression were 7.1 (range 0.2–38.3), 2.6 (range 0.2–28.9) and 3 (range 0–34.2) months, respectively. Among the clinical variables, age, performance status and previous therapy with taxanes were significantly associated with outcomes. Among the molecular markers, TP was found to be associated with progression-free survival. Metronomic therapy is an effective choice for ABC. Young women with a more indolent disease had the greatest benefit from this treatment. TP tumour expression might aid decision making but these findings must be confirmed in larger prospective, properly designed studies. *Anti-Cancer Drugs* 23:326–334 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Breast cancer (BC) is the most frequent malignancy and the second cause of cancer-related death in women [1]. Around 30% of early-stage BCs develop recurrence or distant metastases within the first 2 years after primary treatment [2]. Although mortality has been decreasing steadily in the last 20 years, the mean survival for metastatic disease is still poor, being around 2 years [1–3].

In order to find the best balance between clinical benefit and toxicities, clinical management of metastatic disease has to take into account both patients (age, performance status, comorbidities) and tumour characteristics (hormonal receptors' status, HER2 status, proliferative activity, grading, tumour boarders) [4–6]. Increasing evidences show that cancer genetic profiling can be an accurate instrument for clinical decisions, which helps to define specific risk groups with different prognosis [7] and to predict response to distinct therapeutic options (i.e. hormonal and chemotherapeutic agents, anti-HER2 therapy) [8,9].

The response rate to first-line anthracycline-based, taxane-based or capecitabine-based chemotherapy varies from 25 to 60%. However, the benefit of further lines of treatment is not well established [10]. Chemotherapy, when administered at the maximum tolerated dose, can be difficult to manage and induces severe side effects. Thus, the potential benefit of cancer treatments must be accurately balanced with toxicities, especially in elderly and heavily pretreated patients.

'Metronomic' therapy involves the chronic administration of chemotherapy at a relatively low dose without prolonged drug-free periods [11]. Accumulating evidence suggests that the efficacy of this treatment may rely on antiangiogenic activity, restoration of anticancer immune response and induction of tumour dormancy [11,12]. In advanced breast cancer (ABC), low-dose cyclophosphamide–methotrexate has been shown to be cost-effective and efficient in disease control, yielding an overall response rate of 19% and an overall clinical benefit of 31.7% [13–16]. Interestingly, during metronomic treatment, circulating levels of vascular endothelial growth factor (VEGF) decrease [13], supporting the hypothesis of the potential antiangiogenic activity of this schedule. In patients with ABC receiving metronomic chemotherapy, circulating endothelial cell count after 2 months of

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therapy is a particularly good predictor of disease-free and overall survival after a prolonged follow-up of more than 2 years [17]. Enhanced anticancer activity was documented by combining metronomic chemotherapy with specific antiangiogenic drugs, such as bevacizumab [18–20] and, in animal models, this therapeutic strategy can kill tumour endothelial cells as well as overt cancer cells [21].

Targeting tumour angiogenesis is an effective strategy for limiting cancer growth and metastatization [12]. Bevacizumab, a humanized monoclonal antibody targeting VEGF, combined with different chemotherapeutic agents, provides longer progression-free survival (PFS) in metastatic BC [22–25]. Several drugs targeting angiogenesis have been recently approved for treatment in different solid tumours but no molecular markers predicting the effects of these drugs have been identified or standardized so far.

Thymidine phosphorylase (TP) is an enzyme that catalyses the phosphorylation of thymidine and 2′-deoxyuridine to their respective bases and 2-α-deoxyribose-1-phosphate [26]. It is highly expressed in many human solid tumours and, despite conflicting results, its expression has been correlated with aggressiveness and prognosis in BC [26–29]. According to a recent study, possible reasons for the contrasting results obtained in the evaluation of TP tumour expression could be related to the method of detection [30]. Most approaches for evaluating TP expression in tumour tissues include immunohistochemistry (IHC) and quantitative evaluation of mRNA by PCR (RT-PCR). The lack of a correlation between IHC and RT-PCR may either be due to a difference in what or how these methods measure. In addition, more reproducible results may be obtained using an appropriate system for immunohistochemical staining evaluation [31].

Growing evidence suggests that the mechanisms underlying TP aggressiveness in cancer cells could be related to the stimulation of angiogenesis [26,32]. In particular, TP has been reported to stimulate endothelial cell migration and invasion, possibly by inducing the secretion of a combination of angiogenic factors (IL-8, bFGF and TNF-a) and increasing endothelial cell TP-activity [32]. TP has pro-angiogenic activity-recruiting, but not -activating, endothelial cells [27]. The angiogenic mechanism triggered by TP seems to be driven by the release of its metabolite 2-α-deoxyribose-1-phosphate into the extracellular medium [27,32] but the association of TP-expression with other proangiogenic molecules such as VEGF suggests a possible cooperative role in tumour neovascularization [33]. In addition, TP acts in the salvage cascade of DNA metabolism in response to different types of stress including radiotherapy and chemotherapy (anthracyclines, taxanes, cyclophosphamide, platinum) [26,34] and it catalyses the final step of capecitabine conversion into its active metabolite 5-fluorouracil. In a previous study, we suggested that TP expression in tumour tissue correlates with therapeutic benefit from capecitabine treatment in a cohort of 61 patients affected by metastatic BC pretreated with taxanes (either in combination or in sequence with anthracyclines) [35]. We also proposed a strategy of taxane-induced TP expression that could potentiate the activity of capecitabine [36].

In the present study, we investigated the benefit of metronomic therapy in an unselected, heavily pretreated population of patients with a diagnosis of advanced BC and we explored the possible role of TP and other potential angiogenesis markers as predictors of response to metronomic therapy.

Materials and methods

We retrospectively reviewed the clinical records of 62 patients affected by advanced BC who received chemotherapy with metronomic scheduling for at least one cycle at the University Hospital of Udine between October 2004 and January 2010. All the patients were treated with cyclophosphamide orally at a dose of 50 mg a day continuously and methotrexate orally at a dose of 2.5 mg twice a day on days 1 and 4 every week, according to previous studies with the same metronomic regimen [14,15]. Response to treatment was evaluated according to the Response Evaluating Criteria in Solid Tumours (RECIST version 1.1). Tumour evaluation during the treatment was performed approximately every 2 months.

The following efficacy outcomes were analysed:

(1) Overall survival (OS) (calculated from the date of starting the metronomic treatment to death or the last follow-up),
(2) Progression-free survival (PFS) (calculated from the date of starting the metronomic treatment to first evidence of radiological or clinical progression of disease or death),
(3) Survival post-progression (SPP) (calculated from the date of progression from metronomic treatment to death or the last follow-up).

Histological samples of primary cancer or metastases were evaluated centrally at the Pathology Department of University Hospital of Udine. Immunohistochemistry for HER2 (rabbit polyclonal, Hercept Test, Dako kit), proliferating activity (MIB-1 expression, mouse monoclonal, Ki-67; Dako, Denmark), TP, VEGF and VEGF receptor was carried out on 5-μm-thick paraffin-embedded tissue samples. Endogenous peroxidase activity was blocked by incubating the slides in 0.3% H_2O_2 in absolute methylic alcohol for 8 min. Immunohistochemical reactions were performed using the Immunoperoxidase Super Sensitive Polymer Kit/HRP IHC Detection System; BioGenex (Fremont, California, USA). A positive reaction is indicated by a brown colour. The staining was performed on metastases when available; otherwise, it was performed on the primary tumour. Surgery resections were preferred to biopsies.

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Table 1 Molecular subtype classification

Molecular subtypes	Biological features
Luminal A	ER+ and/or PgR+, HER2− and Ki-67<14%
Luminal B	ER+ and/or PgR+, HER2− and Ki-67 ≥ 14%
Luminal/HER2	ER+ and/or PgR+ and HER2+
HER2 enriched	ER−, PgR− and HER2+
Basal-like	ER−, PgR−, HER− and EGFR+ and/or CK5/6/14+

EGFR, epidermal growth factor receptor.

All cases were further investigated for the expression of hormonal receptors, epidermal growth factor receptor and cytokeratines 5, 6 and 14 to classify the tumours as luminal A, luminal B, luminal/HER2, HER2 enriched or basal like (according to the classification used by Kennecke *et al.* [7]) Table 1.

Thymidine phosphorylase immunohistochemistry

Antigen retrieval was carried out by incubating the samples in EDTA mmol/l pH 8 at 98°C for 30 min. The staining was performed with a primary, anti-mouse antibody anti-TP (clone p-P-GF44C; Abcam, Cambridge, UK) diluted 1 : 50 and incubated overnight at 4°C. TP expression was evaluated on cell cytoplasm according to the Tsuda Scoring System [31]. The intensity of immunoreactivity was scored as 0, 1, 2 or 3, indicating negative, weak, moderate or strong staining, respectively. Then, the percentages of areas of each category (0–3) of staining were estimated and, regardless of positive or negative staining, the category of the largest area of cancer tissue was reported as the representative score for each case. Therefore, for statistical analysis, the TP-expression level for each case was putatively defined as positive if the predominant intensity was 2 or 3.

Vascular endothelial growth factor immunohistochemistry

After the antigen retrieval was performed with citrate buffer 0.01 mmol/l pH 6 at 98°C for 40 min, samples were incubated with a monoclonal anti-mouse antibody (clone 5C3.F8 ab3109; Abcam). Tumour positivity for VEGF was evaluated on cell cytoplasm. The sample was considered positive when more than 50% of the cells had moderate (2+) to strong (3+) cytoplasmic staining.

Vascular endothelial growth factor receptor immunohistochemistry

VEGFR staining was carried out with a polyclonal anti-rabbit antibody (Novus Biologicals Inc., Littleton, Colorado, USA) after antigen retrieval was performed with citrate buffer 0.01 mmol/l pH 6 at 98°C for 40 min. A tumour was considered VEGFR positive when more than 50% of the cells had moderate (2+) to strong (3+) cytoplasmic staining.

Statistical analysis

A univariate analysis was performed using the log-rank test to determine whether the survival outcomes (OS, PFS and SPP) were different across the strata of the following variables: stage at diagnosis, performance status (PS) at the start of the metronomic schedule, sites of metastases, hormonal receptor status, HER2 status, Ki-67 expression, molecular subtype, TP-expression, VEGF-expression, VEGFR-expression, lines and types of chemotherapies (anthracycline-based, taxane-based, cyclophosphamide-based and/or methotrexate-based chemotherapies) and hormonal treatments received before starting the metronomic schedule. The variables that showed a significant difference across strata and were thus associated with survival were included in a multivariate Cox proportional hazards regression analysis. All the analyses were performed using the Statistical Analysis System (SAS; SAS Institute, Cary, North Carolina, USA), release 9.1.

Results

The main characteristics of the patients and disease are presented in Table 2.

The median duration of metronomic treatment was 2.6 months (range 0.3–29), with a median follow-up of 10.5 months (range 0.3–38.3). At the time of the analysis, 58 patients had stopped the treatment because of toxicity ($n = 2$), progression of disease ($n = 46$) or death ($n = 10$). Seventeen patients (27.4%) were still alive and four (6.4%) were still on treatment. After stopping the metronomic schedule, 38 patients (61.3%) received further treatments.

For the whole cohort, the median OS was 7.1 months (range 0.2–38.3), the median PFS was 2.6 months (range 0.2–28.9) and the median SPP was 3 months (range 0–34.2).

The results of the univariate analysis (log-rank test) are reported in Table 3.

The age at initiation of metronomic treatment, evaluated by Cox regression, resulted in statistically nonsignificant hazard ratios (HR) both for PFS (HR = 1.01; 95% confidence interval 0.99–1.03; $P = 0.40$) and OS (HR = 0.99; > 0.97–1.02; $P = 0.67$).

A significant difference in OS (9.4 vs. 20 months; $P = 0.0401$) and SPP (3.7 vs. 10.2 months; $P = 0.0388$) was found between patients receiving or not taxanes before starting the metronomic schedule. On analysing the characteristics of the two groups, we observed a higher proportion of negative prognostic factors in patients who received taxanes-based chemotherapy as first line in the treatment of metastatic disease. The main characteristics of the two groups are presented in Table 4.

Poor PS at the start of the metronomic schedule correlated with poor median OS [12.9 (PS = 0–1) vs. 1.1 months (PS = 2–3); $P < 0.0001$], PFS (2.9 vs. 1.1 months; $P = 0.0018$) and SPP (8 vs. 0 months; $P < 0.0001$).

A shorter median SPP was observed in patients with advanced disease at diagnosis (2.5 vs. 8.9 months;

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Table 2 Patients and disease characteristics at diagnosis and at the start of the metronomic treatment

Characteristics	*N*(=62) (%)
Mean age at starting the treatment (years)	63 (range 37–89)
PS (ECOG) at starting the treatment	
0–1	50 (80.6)
2–3	12 (19.4)
Sites of metastases	
Visceral	46 (74.2)
Bone only	7 (11.3)
Others	9 (14.5)
ER/PgR	
Positive	53 (85.5)
Negative	9 (14.5)
HER2	
Positive	8 (12.9)
Negative	53 (85.5)
Missing	1 (1.6)
Ki-67	
$\geq$ 14%	28 (45.2)
$<$14%	31 (50.0)
Missing	3 (4.8)
Molecular subtype	
Luminal A	27 (43.6)
Luminal B	19 (30.8)
Luminal/HER2	5 (8.0)
HER2 enriched	3 (4.8)
Basal-like	6 (9.6)
Missing	2 (3.2)
TP	
Positive	29 (46.8)
Negative	22 (35.5)
Not valuable	2 (3.2)
Missing	9 (14.5)
VEGF	
Positive	23 (37.1)
Negative	26 (42.0)
Not valuable	4 (6.4)
Missing	9 (14.5)
VEGFR	
Positive	11 (17.8)
Negative	38 (61.3)
Not valuable	4 (6.4)
Missing	9 (14.5)
CHT/HT before metronomic therapy	
Neoadjuvant CHT/HT	6 (9.6)
Adjuvant CHT/HT	49 (79.0)
Palliative CHT/HT	61 (98.4)
Number of lines CHT before metronomic therapy	
$\leq$ 3 lines CHT	24 (38.7)
$>$3 lines CHT	38 (61.3)
Number of lines HT before metronomic therapy	
$\leq$ 3 lines HT	38 (61.3)
$>$3 lines HT	24 (38.7)
AC/T/C/mtx before metronomic therapy	
AC only	53 (85.5)
T only	49 (79.0)
A, T	44 (71.0)
C	44 (71.0)
MTX	30 (48.4)
C, MTX	30 (48.4)

AC, anthracyclines; C, cyclophosphamide; CHT, chemotherapy; ECOG, Eastern Cooperative Oncology Group Performance Status; HT, hormone therapy; MTX, methothrexate; PS, performance status; T, taxanes; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

$P = 0.0193$) and visceral metastases (3.7 vs. 10.6 months; $P = 0.0492$) at the start of the metronomic treatment.

A shorter OS, although only borderline statistically significant, was detected in women with advanced disease at diagnosis (5.4 vs. 12.9 months; $P = 0.0528$), more than two metastatic sites (11 vs. 15 months; $P = 0.0517$) and visceral metastases (11 vs. 19.7 months; $P = 0.0614$).

Among the histopathological and immunohistochemical features, only TP tumour expression was associated with outcomes. Patients with tumour overexpressing TP had shorter PFS (2 vs. 3.3 months; $P = 0.0333$) compared with patients with negative TP expression. Kaplan–Meier curves for the most relevant outcomes are shown in Figs 1–8.

In the multivariate analysis, we observed that all the outcomes from metronomic treatment (OS, PFS, SPP) depend on age at starting the therapy. The benefit from the metronomic schedule decreases with age. Previous chemotherapy with taxanes in women receiving a metronomic schedule was associated with poor OS and SPP and the presence of visceral metastases at the start of the metronomic treatment correlated independently with poor OS.

Results, expressed as hazard ratios, are summarized in Table 5.

Discussion

BC is the most frequent malignant tumour in women and it is the second leading cause of cancer-related death in women [1]. During the last 20 years, mortality has been decreasing steadily because of improvements in diagnosis and treatment [1–3]. In particular, the introduction of hormonal therapies and, recently, of targeted therapies has led to a better control of disease both in the early and in the advanced stages. Despite the well-established efficacy of first-line chemotherapy with or without biological agents, only few data are available on the benefit of further treatments in second-line and third-line therapy [37]. In this setting, data on OS are often difficult to interpret due to the possible influence on the final outcomes of the sequential administration of several different lines of therapy [38]. Thus, there has been increasing interest in survival outcomes such as SPP, which can better indicate the benefit from lines of treatment other than the first-line one [39,40].

The metronomic schedule is based on a continuous, low-dose administration of chemotherapy. In heavily pretreated patients with metastatic BC, metronomic therapy can provide up to 15.7% of prolonged clinical benefit with manageable side effects [15]. Interestingly, in preclinical models, the metronomic schedule could inhibit endothelial cell progenitors and enhance the release of angiogenesis inhibitors [7]. Furthermore, a synergic activity has been demonstrated between the metronomic schedule and antiangiogenic targeted therapies [20]. More recently, baseline circulating endothelial cells and VEGF-A have been shown to predict benefit from metronomic cyclophosphamide and capecitabine plus bevacizumab [41].

Efficacy in limiting tumour neoangiogenesis can be one of the mechanisms by which metronomic therapy controls tumour growth.

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Table 3 Univariate analysis

	PFS		OS		SPP	
Variables	Median values (95% CI)	*P* value	Median values (95% CI)	*P* value	Median values (95% CI)	*P* value
E stage vs. LA + A stage	2.9 (2.5–3.8)	0.28	12.9 (9.4–18.2)	0.05	8.9 (3.7–12)	0.02
	2.2 (1.4–3.2)		5.4 (2–12.4)		2.5 (0–4.2)	
ER ≥ 10% vs. ER < 10%	2.7 (2.3–3.5)	0.44	12.4 (7.7–16)	0.89	5.3 (3.7–10.2)	0.40
	2.5 (1.2–9.4)		5.2 (1.3–nd)		2 (0.4–8)	
PgR ≥ 10% vs. PgR < 10%	2.5 (2.1–3.2)	0.61	12.3 (5.7–13.7)	0.47	5.3 (2.4–10)	0.65
	2.8 (2.3–3.7)		9.4 (4.8–19.7)		3.7 (2–16)	
ER + /PgR + vs. ER– e PgR–	2.7 (2.3–3.5)	0.28	12.3 (6.5–15)	0.47	5.3 (3.6–10.2)	0.87
	2.5 (1.4–9.4)		5.1 (4.2–nd)		2 (0–2.5)	
HER2 pos vs. HER2 neg	3 (0.9–9.4)	0.45	11.4 (1.3–nd)	0.32	9.1 (0.4–nd)	0.54
	2.5 (2.3–3.2)		11.1 (5.7–13.7)		4.2 (2.5–8.9)	
Ki-67 ≥ 14% vs. Ki-67 < 14%	2.6 (2.3–3.5)	0.43	11.1 (5.3–15)	0.34	8 (2.4–16.5)	0.18
	2.6 (2.1–3.7)		9.4 (4.8–32.7)		3.9 (2–8.9)	
Molecular subtype:		0.41		0.66		0.77
LA	2.6 (2.1–5.7)		12.3 (5.7–15)		5.3 (2.5–10.2)	
LB	2.5 (2.3–3.7)		12.4 (4.4–20)		4.2 (2.4–16.5)	
LH	2.8 (0.9–3.7)		11.4 (1.3–nd)		9.1 (0.4–nd)	
H2	9.4 (0.6–nd)		nd (0.6–nd)		0	
BL	2.1 (1.4–3.2)		4.9 (4.2–9.4)		2.3 (2–8)	
TP pos vs. TP neg	2 (1.4–2.8)	0.03	4.6 (2.8–13.7)	0.46	2.4 (0.4–7.1)	0.99
	3.3 (2.4–6.5)		11.1 (6.3–16.7)		5.3 (2.5–10.2)	
VEGF pos vs. VEGF neg	2.5 (2.3–3.4)	0.89	11 (4.6–19.7)	0.32	5.3 (2.4–10.5)	0.22
	2.5 (2–3.2)		6 (3.9–12.3)		2.5 (0.8–5.3)	
VEGFR pos vs. VEGFR neg	2.3 (1.2–3.2)	0.24	5.1 (1.3–19.7)	0.99	2.5 (0.4–16)	0.75
	2.5 (2.1–3.2)		6.5 (4.8–12.4)		3.7 (1.9–5.3)	
≤ 3 lines CHT premetronomic vs. > 3 lines	3.6 (1.7–6)	0.26	15 (4.8–32.7)	0.40	5.3 (2.5–16.5)	0.83
	2.5 (2.4–2.9)		11.4 (6.3–13.7)		4.2 (2.1–10)	
≤ 3 lines HT premetronomic vs. >3 lines	2.5 (2–3.2)	0.47	7.7 (4.6–13.6)	0.45	2.5 (2–8.9)	0.48
	2.8 (2.4–5.6)		12.9 (6.5–19.7)		7.1 (3.9–16)	
AC premetronomic vs. no AC premetronomic	2.6 (2.3–3.2)	0.69	11.1 (5.5–13.7)	0.97	4.2 (2.4–10)	0.72
	5.6 (2.3–6.5)		15 (4.8–16.7)		3.6 (2.5–10.2)	
T premetronomic vs. no T premetronomic	2.5 (2.1–2.9)	0.09	9.4 (4.8–12.9)	***0.04***	3.7 (2.1–8.9)	***0.04***
	5.6 (2.5–9.4)		20 (10–36.7)		10.2 (5.3–34.2)	
C premetronomic vs. no C premetronomic	2.7 (2.4–3.7)	0.46	12.3 (5.7–16.7)	0.17	5.3 (2.4–10.5)	0.31
	2.4 (1.7–3.7)		9.4 (4.8–13.7)		4.2 (2–8)	
MTX premetronomic vs. no MTX premetronomic	2.9 (2.1–3.8)	0.72	12.3 (5.5–16.7)	0.46	5.3 (2.1–12)	0.53
	2.5 (2.3–3.5)		11.1 (4.8–13.7)		4.2 (2.5–10)	
PS (ECOG) 0–1 vs. PS (ECOG) 2–3	2.9 (2.5–3.7)	***0.002***	12.9 (11–18.2)	***<0.0001***	8 (3.8–10.6)	***<0.0001***
	1.1 (0.8–2)		1.1 (0.8–3.3)		0 (0–0.8)	
1 o 2 metastatic sites vs. > 2 metastatic sites	3.3 (2.5–3.8)	0.11	15 (5.5–32.7)	0.05	7.1 (2.1–16.5)	0.18
	2.4 (2.1–2.9)		11 (4.8–13.6)		3.8 (2.4–9.1)	
Visceral metastases vs. not visceral metastases	2.5 (2.3–3.2)	0.26	11 (5.1–12.9)	0.06	3.7 (2.1–8.9)	***0.05***
	3.3 (2.3–3.8)		19.7 (9.4–32.7)		10.6 (3.9–22.4)	
Bone only metastases vs. other sites of metastases	2.9 (2–3.8)	0.69	19.7 (5.3–36.7)	0.19	16 (3.9–34.2)	0.09
	2.6 (2.3–3.4)		11.1 (5.7–13.6)		4.2 (2.5–8.9)	

Bold italic numerals represent the most meaningful results.
AC, anthracyclines; BL, basal-like; C, cyclophosphamide; CHT, chemotherapy; CI, confidence interval; E, early; ECOG, Eastern Cooperative Oncology Group Performance Status; H2, HER2 enriched; HT, hormone therapy; LA, luminal A; LA + A, locally advanced + advanced; LB, luminal B; LH, luminal/HER2; MTX, methothrexate; nd, not determined; OS, overall survival; PFS, progression-free survival; SPP, survival postprogression; T, Taxanes.

Table 4 Main characteristics of the patients who received/did not receive taxanes

Characteristics	No taxanes	Taxanes before metronomic therapy
Number of patients	13	49
Mean age at starting metronomic therapy (years)	72.8	61.2
Locally advanced/advanced disease at diagnosis (%)	15.4	36.7
N + at diagnosis (%)	84.6	81.6
ER/PgR negative (%)	7.7	16.3
HER2 positive (%)	8.3	16.3
Ki-67 >14% (%)	33.3	51.1
>3 lines of CHT before metronomic therapy (%)	15.4	73.5
Visceral metastases at starting metronomic therapy (%)	46.1	81.6

CI, confidence interval; CHT, chemotherapy.

The aim of our study was to determine the clinical and pathological features that could predict prognosis and benefit from metronomic therapy in an unselected, heavily pretreated population of patients with metastatic BC. Furthermore, because of preclinical and translational data suggesting an antiangiogenic mechanism of action of metronomic therapy, we investigated the association between expression of several molecules related to neoangiogenesis and prognosis in the same cohort of patients.

The median OS for the whole group was 7.1 months, which is in line with that of similar heavily pretreated patient populations with ABC. Patients of this cohort received a mean of six different lines of chemotherapy and hormonal treatment before the metronomic sche-

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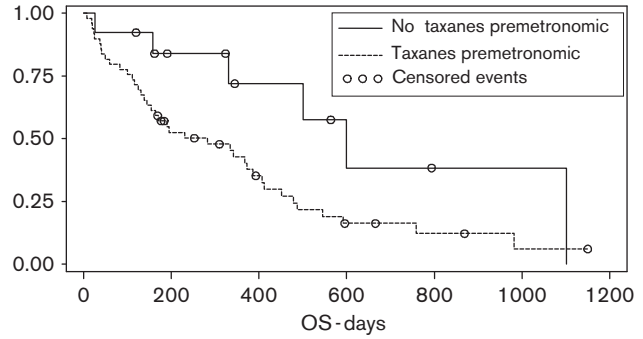
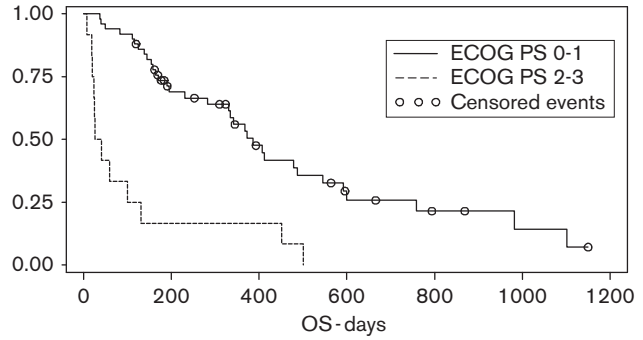
Fig. 1



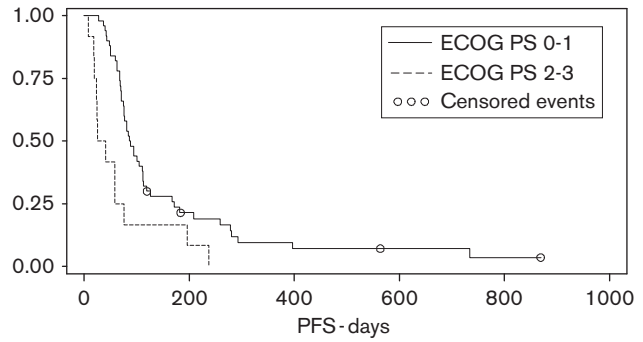
Overall survival (OS) for patients receiving or not taxanes before the metronomic schedule.

Fig. 2



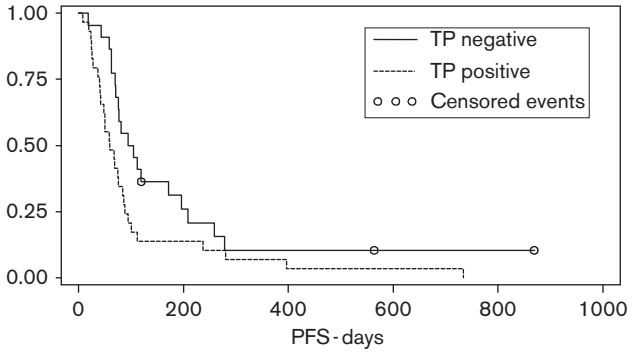
Overall survival (OS) for patients with performance status (PS) 0–1 vs. PS 2–3.

Fig. 3



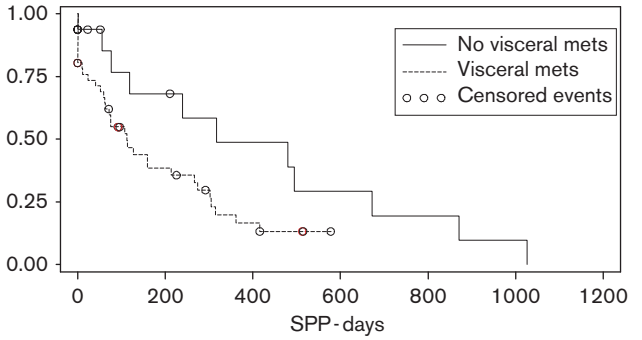
Progression-free survival (PFS) for patients with performance status (PS) 0–1 vs. PS 2–3.

Fig. 4



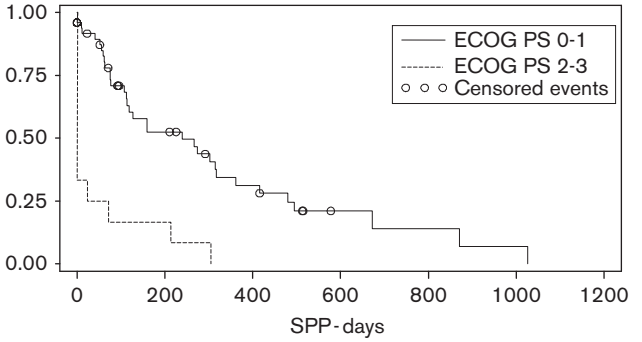
Progression-free survival (PFS) for patients with negative thymidine phosphorylase (TP) vs. positive TP.

Fig. 5



Survival postprogression (SPP) for patients with or without visceral metastases.

Fig. 6



Survival postprogression (SPP) for patients with performance status (PS) 0–1 vs. PS 2–3.

dule. In this setting, SPP provides further information about prognosis by predicting the residual survival time after progression on metronomic therapy. In addition, benefit from potential additional therapy after metronomic therapy is also indicated by SPP. For the whole group of patients included in the study, median SPP was 3 months. The short SPP suggests a very small benefit from metronomic therapy in this setting (heavily pre-

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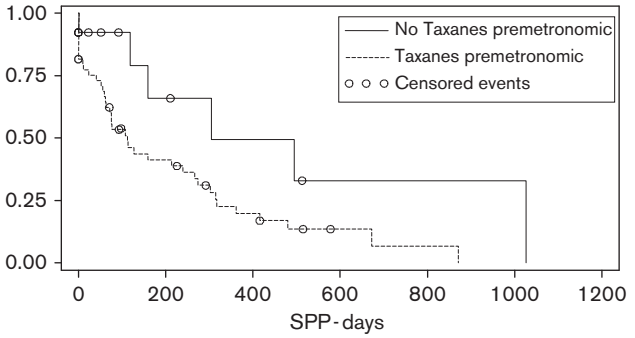
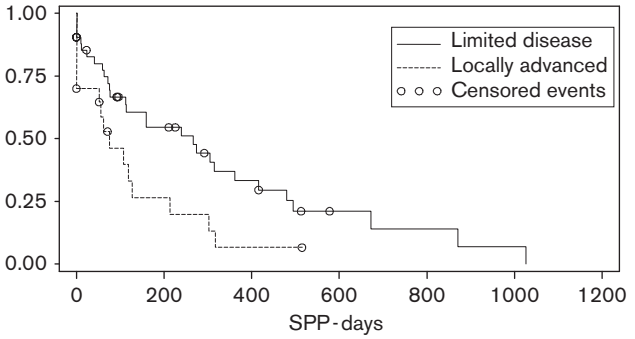
Fig. 7



Survival postprogression (SPP) for patients who received or did not receive taxanes before the metronomic schedule.

Fig. 8



Survival postprogression (SPP) for patients with limited vs. locally advanced disease.

Table 5 Multivariate analysis

Variables	χ^2	*P* value	HR	95% CI
OS				
Age	24.9898	<0.0001	1.215	1.125–1.311
Taxanes premetronomic therapy	6.1904	0.01	12.248	1.702–88.145
PS (ECOG)	0.0549	0.81	1.218	0.234–6.335
>2 metastatic sites	0.5971	0.44	2.030	0.337–12.239
Visceral metastases	16.7794	<0.0001	12.248	1.702–88.145
Bone-only metastases	0.4454	0.50	0.647	0.180–2.323
PFS				
Age	36.5908	<0.0001	1.186	1.122–1.253
TP status	0.1370	0.71	0.835	0.321–2.173
PS(ECOG)	2.844	0.09	4.499	0.784–25.832
SPP				
Age	9.4496	0.002	1.068	1.024–1.114
Stage at diagnosis	1.1875	0.28	1.761	0.636–4.872
Taxanes premetronomic therapy	4.0894	0.04	5.012	1.051–23.905
PS (ECOG)	0.0176	0.89	0.914	0.244–3.43
>2 metastatic sites	0.8013	0.37	1.902	0.465–7.778
Visceral metastases	1.6943	0.19	5.850	0.409–83.601
Bone-only metastases	0.5191	0.47	3.035	0.148–62.160

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; PS, performance status, PFS, progression-free survival; SPP, survival postprogression; TP, thymidine phosphorylase.

treated metastatic breast cancer). To our knowledge, these are the first data published on SPP in this setting. This information may be useful for the design and interpretation of future studies. In fact, where SPP is low, the probability to show differences in OS between the experimental and the control arms in a randomized clinical trial is more probable, making OS an optimal endpoint.

Only two patients discontinued the therapy because of side effects, confirming the favourable toxicity profile of this schedule and the low impact on quality of life.

Age at start of the metronomic schedule correlates negatively with all survival outcomes, suggesting that benefit from chemotherapy should be carefully evaluated in elderly patients. This evidence can be partially justified by better organ function and tolerance to treatment toxicities in young patients [38].

Notably, women receiving taxanes before the metronomic schedule had shorter OS and PFS compared with those who did not. However, in the two groups, we observed a nonuniform distribution of several disease and clinical characteristics (mean age at start of the metronomic schedule, stage at diagnosis, hormonal status, HER2 status, proliferating activity, sites of metastases at the start of metronomic treatment, number of lines of chemotherapy received), which might have affected the final result. These data overall suggest a more aggressive disease in patients receiving taxanes at an early stage. However, prolonged OS and SPP for patients who had not had a taxane-based treatment before metronomic therapy would suggest that more indolent disease may influence the clinical decision-making process. In other words, clinicians may prefer to use taxanes in first lines when prognostic factors are predominantly unfavourable.

In the present study, we found that a high expression of TP was associated with shorter PFS. The exact molecular mechanisms underlying the aggressiveness of cancer cells that express TP are still unclear. Two different, but possibly overlapping actions have been proposed. First, TP may confer a higher invasive property to cancer cells, enhancing their metastatic potential [26]. Second, a growing body of preclinical evidence suggests that TP is related to an increased angiogenesis [26,32]. The results of our study, although preliminary, did not support the hypothesis that metronomic therapy may contrast the proangiogenic activity of TP. In addition, we have not indentified other biomarkers that may potentially be predictors of outcome.

Interestingly, a previous study showed that serum levels of HER2 and epidermal growth factor receptor were associated with probability of response, PFS and OS in patients treated with metronomic chemotherapy [42].

Several of the published studies assessing predictive or prognostic factors of metronomic chemotherapy share some limitations; the small number of patients, a nonrandomized design and retrospective analysis are the

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most commonly observed limitations. In our study, all these drawbacks were present and the results have to be interpreted with caution. In addition, due to the limited availability of tissue from metastases, the analyses of biomarkers were mainly performed on primary tumour samples. One may expect differences between primary cancers and corresponding metastases, especially in terms of angiogenesis.

In conclusion, our study supports the value of metronomic chemotherapy as an option in the sequence of treatments in patients with advanced BC. The study provided information about different measures of efficacy (PFS, OS, SPP) that may be useful in interpreting and designing future clinical trials. Among the biomarkers, TP expression was associated with a worse outcome, but this finding must be considered as hypothesis-generating. Translational research aimed at further investigation of the prognostic role of TP as well as the potential antiangiogenic effect of TP-directed agents (e.g. capecitabine) is warranted [43].

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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