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Increased mean corpuscular volume of red blood cells predicts response to metronomic capecitabine and cyclophosphamide in combination with bevacizumab

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ABSTRACT

Background: There is an urgent need for the identification of commonly assessable predictive factors in the treatment of patients with metastatic breast cancer.
Methods: During the course of a treatment including low dose metronomic oral cyclophosphamide and capecitabine plus i.v. bevacizumab (plus erlotinib in one third of the patients) for metastatic breast cancer, we observed that a relevant number of patients developed repeatedly elevated levels of mean corpuscular volume (MCV) of red blood cells without a significant fall in hemoglobin levels. We conducted a retrospective analysis on these 69 patients to evaluate if the increase in MCV could be associated to tumor response.
Results: During the course of treatment 42 out of 69 patients (61%) developed macrocytosis. Using Cox proportional hazards modeling that incorporated macrocytosis (MCV≥100 fl) as a time-dependent covariate, macrocytosis resulted in a halved risk of disease progression (HR 0.45; 95% CI, 0.22–0.92, *p*-value 0.028). In a landmark analysis limited to patients with no sign of progression after 24 weeks of treatment, median time to progression was 72 weeks (48 weeks after landmark) in patients who had developed macrocytosis, and 43 weeks (19 weeks after landmark) in patients who had not ($p = 0.023$).
Conclusion: Macrocytosis inversely related to risk of disease progression in patients treated with metronomic capecitabine plus cyclophosphamide and bevacizumab for metastatic breast cancer. This finding may be explained through thymidylate synthase inhibition by capecitabine. Whether bevacizumab has a role in determining macrocytosis, similarly to what happens with sunitinib, has to be further investigated. If other studies will confirm our findings, macrocytosis might be used as an early marker of response during metronomic treatment with capecitabine and cyclophosphamide with or without bevacizumab.

Introduction

Metastatic breast cancer is considered a chronic disease, where the aim of treatment is the improvement of quality of life and prolongation of survival. Developments in therapeutic interventions for metastatic breast cancer have led to improvements in time to disease progression, time to treatment failure, quality of life, and overall survival.[1–5] Research is now focused on developing novel treatment strategies that might be as effective but less toxic than standard chemotherapy. These new agents may have an important role in the management of patients with metastatic breast cancer due to their favorable safety profiles and lack of cumulative toxicity.

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Angiogenesis is a key process for tumor development and a relevant target for tumor control.[6] Tumor angiogenesis is regulated by a number of stimulatory and inhibitory molecules, and the vascular endothelial growth factor (VEGF) family of stimulators is the main player in many tumor types, promoting endothelial cell survival, division, migration, as well as vascular permeability and mobilization of immature bone-marrow-derived endothelial progenitor cells into the peripheral circulation.[7] This suggests the need for combined inhibition of multiple pathways or the sequential addition of different antiangiogenic agents, such as bevacizumab, a humanized monoclonal antibody directed against VEGF, as strategies for long term tumor control.[8]

The term 'metronomic' chemotherapy refers to the frequent, even daily, administration of chemotherapeutics at doses significantly below the maximum tolerated dose, with no prolonged drug-free breaks.[9] Many chemotherapeutic agents have been shown to exert cytotoxic effects not only on tumor cells but also on the endothelial cells of tumor microvasculature. This antiangiogenic activity seems prominent with the protracted exposure to low doses of chemotherapeutics, compared with their cyclic administration at the maximum tolerated dose.[10]

The choice of treatment in metastatic breast cancer is usually based on disease characteristics (i.e., estrogen and progesterone receptor status, HER2 overexpression and/or amplification) and patients' characteristics (in particular patients' age, the presence of symptoms, and sites of metastases), and ultimately on patients' preferences. The identification of commonly assessable predictive factors would be extremely useful in the clinical practice, since the single patient could be spared the toxicity of a treatment if this is found to be ineffective to cure her disease.

During the course of a treatment including low dose metronomic oral cyclophosphamide (Endoxan®, Baxter, 50 mg daily) and capecitabine (Xeloda®, Roche, 500 mg 1 tablet thrice daily) plus i.v. bevacizumab (Avastin®, Roche, 10 mg/kg i.v. every 14 days or 15 mg/kg i.v. every 21 days) for metastatic breast cancer, we observed that a relevant number of patients developed repeatedly elevated levels of mean corpuscular volume (MCV) of red blood cells without a significant fall in hemoglobin levels. This finding was previously described by Wenzel et al[11] in 154 advanced cancer patients receiving capecitabine (2500 mg/m2/day for 14 days every 21 days) either as monotherapy or in combination with other antineoplastic agents. A statistically significant increase in MCV (without other hematologic abnormalities or clinical symptoms) could be observed within 9 weeks ($p < 0.0001$). Higher MCV values were seen in patients with tumor remission or stable disease than in patients with tumor progression, but the difference was not statistically significant.

We conducted the present investigation to evaluate if the increase in MCV could be associated to tumor response in metastatic breast cancer patients treated with metronomic chemotherapy in association with antiangiogenic therapy.

Patients and methods

Patients

A total of 69 patients with histologically proven advanced breast cancer were included in the analysis. Patient characteristics at baseline are shown in Table 1.

Forty-six patients received 10 mg/kg bevacizumab (Avastin®, Roche, i.v. every 14 days) in combination with oral cyclophosphamide (Endoxan®, Baxter, 50 mg 1 tablet daily at 9 AM), plus oral capecitabine (Xeloda®, Roche, 500 mg 1 tablet thrice daily after meals) within the context of a phase II trial, as previously described.[12] Twenty-three patients received 15 mg/kg bevacizumab (Avastin®, Roche, i.v. every 21 days) in combination with oral cyclophosphamide (Endoxan®, Baxter, 50 mg 1 tablet daily at 9 AM), oral capecitabine (Xeloda®, Roche, 500 mg 1 tablet three times daily after meals), and oral erlotinib (Tarceva®, Roche, 100 mg 1 tablet once daily either at least 1 h before or 2 h after meals), alone if HER-2 negative ($n = 18$) or in combination with 6 mg/kg trastuzumab (Herceptin®, Roche, i.v. every 21 days, with a loading dose of 8 mg/kg at the first administration) if HER2-positive ($n = 5$) within the context of a second phase II trial (Montagna et al., submitted).

In both trials, response to treatment was assessed every 8 weeks by repeating the same exams used at baseline (either conventional/spiral CT scan or conventional MRI, plus clinical measurements or

Table 1
Patients characteristics at baseline ($N = 69$).

Characteristic	No.	%
Regimen		
BEX	46	67
BEXE	18	26
BEXET	5	7
Age, years		
Median	55	
Range	32–75	
Body weight, kg		
Median	65	
Range	45–99	
Menopausal status		
Premenopausal	24	35
Postmenopausal	45	65
Metastatic sites[a]		
Bone	29	42
Lung	20	29
Liver	24	35
Lymph nodes	25	36
Pleura	8	12
Others	10	14
No. of metastatic sites		
1	34	44
2	24	35
≥3	11	16
Tumor hormone receptor status[b]		
ER positive and PgR positive	15	22
ER positive and PgR negative	29	42
ER negative and PgR negative	25	36
HER2/neu status		
Absent	27	39
1+	29	42
2+	7	10
3+	6	9
Prior neoadjuvant therapy		
No	55	80
CT	9	13
CT/HT	5	7
Prior adjuvant therapy		
No	20	29
CT	12	17
HT	11	16
CT/HT	26	38
Prior therapy for metastatic disease		
No	33	48
CT	3	4
HT	17	25
CT/HT	14	20
CT/HT/trastuzumab	2	3
No. of prior metastatic CT regimens		
0	50	72
1	11	16
≥2	8	12
Prior anthracycline	37	54
Prior taxane	16	23

Abbreviations: BEX = Bevacizumab, Endoxan, Xeloda; BEXE = Bevacizumab, Endoxan, Xeloda, Erlotinib; BEXET = Bevacizumab, Endoxan, Xeloda, Erlotinib, Trastuzumab; ER = estrogen receptor; PgR = progesterone receptor; CT = chemotherapy; HT = hormone therapy.

[a] Multiple sites possible.

[b] Positive: ≥10%.

ultrasound of superficial palpable breast nodes, lymph nodes and subcutaneous lesions). Response was assessed using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee,[13] in which changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used. Both protocols did not require an independent validation of response assessments.

Toxicities were graded using the National Cancer Institute Common Terminology Criteria of Adverse Events (CTCAE version 3).[14] Bevacizumab was to be permanently discontinued in patients who developed GI perforation, wound dehiscence requiring medical intervention, serious bleeding, a severe arterial thromboembolic event, nephrotic syndrome, or hypertensive crisis. Temporary suspension of bevacizumab was recommended in patients with evidence of moderate to severe proteinuria pending further evaluation and in patients with severe hypertension that was not controlled with medical management. Cyclophosphamide and capecitabine were reduced in case of grade≥2 hematologic toxicity, cystitis, GI toxicity, or hand-foot syndrome. To achieve a 50% dose reduction, cyclophosphamide was administered as one 50-mg tablet every other day, and capecitabine was administered as one 500-mg tablet once daily alternated with one 500-mg tablet twice daily.

During the course of both trial treatments, we observed that a relevant number of patients developed repeatedly elevated levels of MCV of red blood cells without a significant fall in hemoglobin levels. We therefore conducted a retrospective analysis on the pooled 69 patients to evaluate if the increase in MCV could be associated to tumor response. The retrospective analysis was conducted at the European Institute of Oncology, Milan, Italy. Prior to each cycle of therapy, a complete blood cell count including the red cell indices MCV, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was performed both with the Dasit XE 2100 (Italy) and the Abbot Cell-Dyn Sapphyre (USA) in all patients. For the purposes of the study, macrocytosis was defined as a MCV greater than or equal to 100 fl.

Statistical analysis

A bivariate linear mixed model for longitudinal data[15] was used to analyze the time courses of MCV and hemoglobin levels during the first 24 weeks of treatment and to estimate their correlation. The correlation coefficient ρ was calculated taking into account all available information over the course of observation and dealing with intrasubject correlation. Only patients remaining in the study for at least 24 weeks ($N = 46$) were considered, in order to satisfy the missingness at random assumption[16] required for longitudinal mixed models.

Time to disease progression (TTP) was calculated from the start of treatment to the time of disease progression. Cox proportional hazards regression model was used to determine the association between macrocytosis and TTP. A time-dependent indicator of macrocytosis, switching from '0' to '1' at the time of macrocytosis (MCV≥100 fl) onset, was included in the model. This indicator was kept constant even if the value of MCV returned below 100. The use of macrocytosis as a time-dependent covariate enables accounting for "survivor" bias (i.e., a patient not observed long enough to show macrocytosis).

Furthermore, to account for this bias, a landmark analysis limited to patients with no sign of progression after 24 weeks of treatment was performed. Time to progression was evaluated in patients who had developed macrocytosis and patients who had not, using the Kaplan–Meier estimates of the survival curves. The log-rank test was used for the comparisons of survival curves.

All analyses were carried out with the SAS software (SAS Institute, Cary, NC). All tests were two-sided.

Table 2
Mean corpuscular volume and mean hemoglobin concentration during treatment.

Weeks since start of treatment	No.	Mean corpuscular volume (fl)	Hemoglobin concentration (g/dl)	Presence of Macrocytosis
		Mean (Std. Dev)	Mean (Std. Dev)	%
0	69	88.19 (4.47)	13.15 (1.05)	0%
6	65	90.56 (4.59)	13.19 (0.88)	5%
12	61	95.73 (5.60)	13.02 (0.98)	21%
18	52	100.06 (6.88)	12.94 (1.03)	54%
24	46	101.57 (7.22)	13.15 (1.03)	61%

Results

Prior to the first cycle of treatment, there were no abnormalities in red blood cells, white blood cells and platelets, respectively. At baseline, mean (Standard Deviation) hemoglobin levels (normal range 12–16 g/dl) were 13.1 (1.05) g/dl and MCV (normal range 80–99 fl) was 88.2 (4.5) fl.

During the course of treatment, MCV increased significantly ($p < 0.001$), with 42 out of 69 patients (61%) developing macrocytosis, while hemoglobin levels remained stable ($p = 0.27$) (Table 2). The two trends were not significantly correlated (Fig. 1. $\rho = -0.19$, $p = 0.28$). Severe leukopenia or thrombocytopenia did not occur in any of the patients. We determined vitamin B12 and folic acid levels only in those patients who developed moderate anemia, but no significant alterations in these parameters could be observed during therapy.

Using Cox proportional hazards modeling that incorporated macrocytosis (MCV≥100 fl) as a time-dependent covariate, macrocytosis resulted in a halved risk of disease progression (HR 0.45; 95% CI, 0.22–0.92, *p*-value 0.028).

In a landmark analysis limited to the 46 patients with no sign of progression after 24 weeks of treatment, median time to progression was 72 weeks (48 weeks after landmark) in patients who had developed macrocytosis, and 43 weeks (19 weeks after landmark) in patients who had not ($p = 0.023$) (Fig. 2A). The risk of progression at one year (28 weeks after landmark) was 29% (95% CI 15%–51%) in patients who had developed macrocytosis and 57% (95% CI 35%–81%) in patients who had not. This association was even stronger than the one considering the response to treatment during the first 24 weeks and progression ($p = 0.19$) (Fig. 2B).

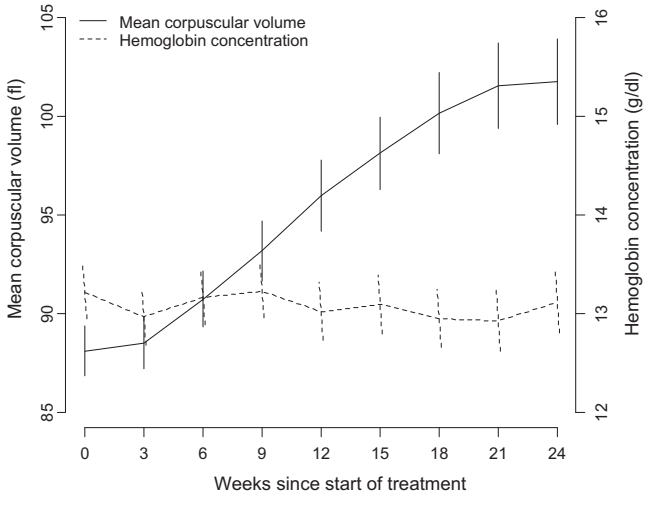

Fig. 1. Mean Corpuscular Volume (left axis) and Hemoglobin Concentration (right axis) observed during the first 24 weeks of treatment (means and 95% confidence intervals observed at different time points). Only patients remaining in the study for at least 24 weeks ($N = 46$) were considered in this analysis.

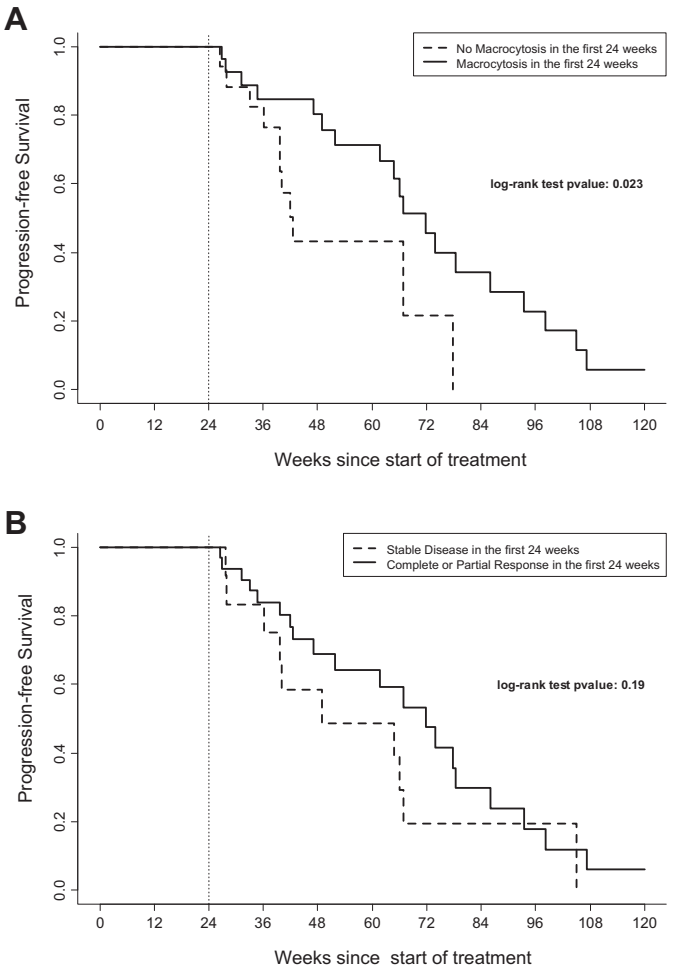

Fig. 2. (A) Landmark analysis of time to progression according to Macrocytosis observed in the first 24 weeks of treatment. All patients remaining in the study for at least 24 weeks ($N = 46$) were categorized according to the presence or absence of Macrocytosis. (B) Landmark analysis of time to progression according to best response to treatment observed in the first 24 weeks. All patients remaining in the study for at least 24 weeks ($N = 46$) were categorized according to the presence or absence of complete or partial response.

Among the 23 patients not included in the landmark analysis, 7 (30%) developed macrocytosis. Among the 46 patients included in the landmark analysis, 18 (39%) did not develop macrocytosis during the first 24 weeks of treatment.

We evaluated the association between macrocytosis and toxicities. Differences were found between the number of adverse events (any grade) observed in patients who developed and who did not develop macrocytosis for anemia (24% vs 4%, $p = 0.04$), hypertension (50% vs 22%, $p = 0.03$) and proteinuria (29% vs 7%, $p = 0.04$).

Discussion

During the course of treatment with metronomic capecitabine plus cyclophosphamide and i.v. bevacizumab for metastatic breast cancer, we observed that a relevant number of patients developed elevated levels of mean corpuscular volume of red blood cells without a significant fall in hemoglobin levels.

In a retrospective review on 76 metastatic breast cancer patients receiving standard 21-day cycles of oral capecitabine therapy, MCV increased in a dose-dependent and time-dependent manner during chemotherapy, with 57% of study patients developing macrocytosis (MCV >100 fl) while on capecitabine.[17]

In a report by Sun et al.,[18] 28 patients with GI malignancies at different stages received continuous, low dose, maintenance capecitabine. MCV was identified as a potential biomarker of adequate dosing of capecitabine when given below its MTD, however elevated MCV did not predict response to maintenance therapy.

Wenzel et al.[11] observed a statistically significant increase of MCV (without other hematologic abnormalities or clinical symptoms) in 154 advanced cancer patients receiving capecitabine (2500 mg/m2/day for 14 days every 21 days) either as monotherapy or in combination with other antineoplastic agents within 9 weeks ($p < 0.0001$) which was probably due to the 5-FU-induced TS inhibition in erythroid precursor cells.

Capecitabine is a fluoropyrimidine carbamate which is selectively activated after oral administration to 5-fluorouracil (5-FU) by a sequential triple enzyme pathway in liver and tumor cells. In the first step, capecitabine is hydrolyzed by carboxylesterase in the liver to 5-deoxy-5-fluorocytidine (5-DFCR). In the second step, cytidine deaminase in the liver and/or tumor tissue, converts 5-DFCR to 5-deoxy-5-fluorouridine (5-DFUR). The third and last step occurs at the tumor site by the tumor-associated angiogenic factor thymidine phosphorylase (TP), metabolizing 5-DFUR to 5-FU.[19–22] Therefore, 5-FU is preferentially generated in tumor tissue when compared with normal body tissue.

The cytotoxic action of 5-FU is mostly based on the inhibition of thymidylate synthase (TS). This effect is mediated by the 5-FU metabolite 5-fluoro-2-deoxyuridine-5–monophosphate (FdUMP), which blocks the de novo synthesis of thymidylate (dTMP) by forming a ternary complex with TS and the essential co-factor 5,10-methylenetetrahydrofolate (CH2-THF) leading to a defective DNA synthesis.[23,24] Whenever the formation of cell DNA from thymidylate is slowed down, the prolonged cell cycle allows excess synthesis of RNA and other cytoplasmic components including hemoglobin, leading to the increased size of red blood cells in megaloblastic anemia.[25] This can be the result of severe deficiencies of vitamin B12 and folic acid, as well as capecitabine treatment as a result of the inhibition of TS in erythroid precursor cells. The consistent finding of macrocytosis during capecitabine treatment, both when given at standard doses and at metronomic doses, may suggest that there is good pharmacodynamic evidence of adequate capecitabine dosage even when it is given at daily low dose.

Bevacizumab is a humanized monoclonal antibody directed against VEGF. There are no data on the possible role of bevacizumab on the development of macrocytosis. In patients with metastatic renal cell carcinoma, however, macrocytosis was a common occurrence after treatment with sunitinib but not sorafenib, two small molecules that inhibit the vascular endothelial growth factor and related receptors.[26] Macrocytosis was also noted in 42% of gastrointestinal stromal tumors (GIST) patients receiving imatinib, a small molecule that has antitumor effect through inhibition of c-KIT, which is constitutively activated in GIST.[27] Imatinib and sunitinib may lead to macrocytosis through c-KIT inhibition,[28] although the precise mechanisms of c-kit–mediated macrocytosis require further investigation. Sorafenib has much weaker inhibitory activity against c-kit, therefore this could explain why macrocytosis was not observed with sorafenib treatment.

In our analysis, the onset of macrocytosis inversely related to risk of disease progression (Hazard Ratio: 0.45; 95% CI: 0.22–0.92, p-value 0.028) in 69 patients treated with metronomic capecitabine plus cyclophosphamide and i.v. bevacizumab for metastatic breast cancer.

In the paper by Wenzel et al.,[11] higher MCV values were seen in patients with tumor remission or stable disease rather than in patients with tumor progression, but the difference was not statistically significant. This might be due first of all to the different tumor types and various treatment schedules considered in the

paper, as compared to our homogeneous cohort of metastatic breast cancer patients. Secondly, the capecitabine schedule considered in the paper was the standard one (2500 mg/m2/day for 14 days every 21 days) while in our report it was a metronomic one (1500 mg/day continuously). Further investigations should be performed to assess whether different schedules of capecitabine may result in different duration of inhibition of TS, similarly to the different mechanism of action of i.v. bolus 5-FU and continuous infusion 5-FU. Given the absence of rest periods during treatment without an opportunity to repair DNA and recover function, the metronomic schedule might exert a permanent inhibition of TS.

TS polymorphism in peripheral blood cells may be used as a surrogate for intratumoral TS.[29] Our finding support the hypothesis that TS inhibition in erythroid precursor cells corresponds with potent TS inhibition in tumor cells.[11]

We observed that patients who developed macrocytosis had a higher risk of developing anemia (24% vs 4%, $p = 0.04$), hypertension (50% vs 22%, $p = 0.03$) and proteinuria (29% vs 7%, $p = 0.04$) than patients who did not develop macrocytosis. Since we assessed the association of macrocytosis with 34 possible adverse events, these results should be taken with caution. In fact, in the presence of multiple comparisons, the risk of reporting false positive results is much higher than the 5% threshold usually considered in defining statistical significance.

In conclusion, macrocytosis significantly predicted tumor response in patients treated with metronomic capecitabine plus cyclophosphamide and i.v. bevacizumab for metastatic breast cancer. These findings may be explained through TS inhibition by capecitabine and likely portray macrocytosis as a pharmacodynamic marker of capecitabine efficacy which is associated with clinical outcome.

Whether bevacizumab has a role as a concomitant factor determining macrocytosis, similarly to what happens with sunitinib, has to be further investigated. If confirmed by other studies, our findings may support the role of macrocytosis as an early surrogate marker of response during metronomic treatment with low dose oral capecitabine and cyclophosphamide with or without bevacizumab.

Conflict of interest statement

None declared.

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