Case Report

Long term complete response in patient with liver metastases from breast cancer treated with metronomic chemotherapy

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ABSTRACT

Background. Preclinical studies have shown that several chemotherapeutic agents at low doses, may affect the vascular system. Here we report a case of a patient with long term control of the disease by metronomic chemotherapy.

Case presentation. A 62 years old woman underwent left mastectomy in July 2007.
For the hepatic recovery she performed first-line chemotherapy with doxorubicin plus paclitaxel every 21 days. The computer tomography (CT) revaluation after the sixth cycle showed a partial response to treatment. It was decided to stop the treatment with doxorubicin and paclitaxel, and to start a metronomic therapy with cyclophosphamide 50 mg daily orally and Methotrexate 2.5 mg twice daily, two days a week. After six months of this maintenance treatment, the CT scan showed a complete response. We evaluated the expression of vascular endothelial growth factor receptor 2 (VEGFR2) on histological sections of the primary tumor of our patient, with evidence of overexpression of the receptor. The metronomic treatment is still ongoing, and after 60 months the patient maintains a complete response.

Conclusion. This clinical case also highlights how suitable metronomic chemotherapy can be as maintenance therapy, allowing long-term treatment with no relevant toxicity. This case suggests that the level of VEGFR2 are predictive of best response to antiangiogenetic therapy.

Key words: Long term complete response, metronomic chemotherapy, breast cancer.

Introduction

Most cytotoxic drugs primarily target DNA, either directly or indirectly. This action results in death of tumor cells, particularly those that are actively proliferating, often with a directly proportional dose-response relationship.

Preclinical studies have shown that several chemotherapeutic agents at low doses, devoid of any effect on tumor cells, may affect the vascular system, targeting the rapid proliferating tumor associated endothelial cells[1].Therefore cytotoxic agents, when administered at low doses on a frequent or continuous "metronomic" schedule, exert antiangiogenic activity with low toxicity[2].

Low doses of methotrexate (M) have been shown to inhibit endothelial cell proliferation in vitro, as well as neovascularization induced by vascular endothelial growth factor in the rabbit cornea assay in vivo[3].

A metronomic administration of cyclophosphamide (C) in mouse models has been shown to inhibit the growth of tumors whose cells had developed resistance against the same drug administered with the conventional schedule. In this experimental model, the apoptosis of vascular endothelial cells preceded that of tumor cells, thus implying an action primarily on tumor vasculature [4].

Two clinical studies have shown that the metronomic administration of low doses of C and M can induce tumor regression in about 20% of patients with advanced breast cancer, with an overall clinical benefit (objective responses or stable disease lasting at least 24 weeks) of 30-40% [5,6]. The percentage of complete responses is about 3%. In both studies, the therapy was well tolerated.

A combined analysis of the two studies has shown a prolonged clinical benefit (≥12 months) in 15.7% of the patients (95% confidence interval 9.9-21.4%), with median time to progression for patients with prolonged clinical benefit of 21 months (range 12-37+ months)[7].

Here we report a case of a patient with solitary liver metastasis from a triple negative breast cancer, with overexpression of the vascular endothelial growth factor receptor 2 (VEGFR2), who achieved long term control of the disease by metronomic chemotherapy.

Case report

A 62 years old woman underwent left mastectomy in July 2007. Histological examination showed a poorly differentiated infiltrating ductal carcinoma of 6 cm of maximum diameter, with metastases in 4 out of 22 axillary nodes. Stage IIIA, pT3 pN2a Mx. Immunohistochemical studies showed no expression of estrogen and progestin receptors and a proliferating index MIB1 of 60%, HER2 was non-amplified at fluorescent in situ hybridization.

The previous medical history was unremarkable, and the patient was taking no medications.

A preoperative chest X-ray was normal. After surgery, she completed the staging examinations with abdomen ultrasound and bone scan. The ultrasound of the abdomen showed a hypoechoic round lesion of the sixth hepatic segment, highly suspicious for metastases. The presence of liver metastases was confirmed by a CT scan (Figure 1A) and a PET (Figure 1B). The lesion measured 20 mm, and had a standardized uptake value of 5.6.

She performed first-line chemotherapy with doxorubicin plus paclitaxel every 21 days, for a total of 6 cycles from August 2007 to December 2007. The CT revaluation after the sixth cycle showed a partial response to treatment. The lesion measured 6 mm (Figure 1C).

In view of the response obtained and the number of cycles performed, it was decided to stop the treatment with doxorubicin and paclitaxel, and to start a metronomic therapy with cyclophosphamide 50 mg daily orally and Methotrexate 2.5 mg twice daily, two days a week (Monday and Tuesday).

After six months of this maintenance treatment, the CT scan showed a complete response. (Figure 1D). A subsequent PET scan, performed after 21 months from start of maintenance therapy, was completely negative.

The metronomic treatment is still ongoing, and after 60 months the patient maintains a complete response (Figure 1E), with no side effects. The revaluation occurs periodically with either CT or ultrasound of the abdomen and chest X-ray.

Immunohistochemistry

Four-micrometer sections of the tissue samples were mounted on positive-charged slides (BioOptica, Milan, Italy), deparaffinised with xylene, rehydrated with EtOH, and the endogenous peroxidase activity blocked by 3% hydrogen peroxide solution. Antigens unmasking was performed by citrate buffer at ph 6 for 30 minutes at 98.5°C.

The sections were cooled in citrate buffer for 30 minutes, and washed in distilled water.

The staining was performed by using the automated Dako Autostainer (Dako Corporation, Carpinteria, CA, USA).

After the sections were incubated for 1 h at room temp with primary antibody anti VEGFR2 (Imgenex Ab -951) diluted in 1:75 in diluent Dako according the manufacturer instructions.

The sections were washed with PBS, incubated with universal biotinylated secondary antibody, rinsed in PBS, and incubated with streptavidin-peroxidase conjugate (LSAB+kit; DAKO) for 15 minutes. Sections were rinsed again in PBS and antibody binding was detected by staining with diaminobenzidine/hydrogen peroxidase chromogen solution (DAB+liquid substrate–chromogen solution; DAKO Corporation). Finally, sections were rinsed in deionised water, counterstained blue by Mayer's Haemalum, and mounted in Eukitt (Bio Optica, Milan, Italy).

Conclusions

Currently, treatment of metastatic breast cancer is essentially palliative, and is chosen depending on the hormonal receptor status and the presence or absence of amplification of HER2. For triple negative tumors, the therapeutic choices are currently limited to chemotherapy and bevacizumab.

The value of maintenance therapy, administered to patients who did not experience disease progression after a line of systemic treatment, is still debated, although a meta-analysis showed that longer first-line chemotherapy duration is associated with marginally longer overall survival and a substantially longer progression-free survival[8]. Maintenance therapy is required to be effective in controlling disease progression and to be well tolerated. Metronomic chemotherapy has the potential to fulfill these requirements in a number of clinical conditions, and is suitable also for patients with triple negative breast cancers, who lack specific targeted therapies.

The concept of metronomic delivery of chemotherapy has become relevant for the treatment of different types of cancer, among which breast cancer, prostate cancer[9], sarcomas[10] and melanoma[11]. Its effective use would be fostered by surrogate markers of antiangiogenic activity and predictors of response.

Measurement of circulating endothelial progenitor cells in peripheral blood is the more promising[12]. Another potential marker is thrombospondin-1 (TSP-1), which is up-regulated by low dose metronomic cyclophosphamide[13], inhibiting endothelial cell proliferation and survival. Clinical studies of metronomic chemotherapy have shown reduction of serum VEGF levels, more evident in responders but present also in non-responder [5,6], although its clinical relevance and the contribution of platelet-derived VEGF remains unknown.

Molecules related to tumor vascularity, such as VEGFR2, are being studied. The VEGF family of angiogenesis stimulators is the main player in breast cancer angiogenesis[14], and VEGFR2 is the receptor mainly involved in both physiological and pathological angiogenesis. Apart from its relevance for drugs directly targeting VEGFR2 itself, its expression could have a broader role in predicting the activity of angiogenesis inhibitors.

We evaluated the expression of VEGFR2 on histological sections of the primary tumor of our patient, with evidence of overexpression of the receptor (Figure 2). Preclinical work has shown highly heterogeneous expression of the receptor in colon cancer and melanoma xenografts, but no clear effect on response to metronomic cyclophosphamide in these models[15], although the authors suggest that response to metronomic therapy could be cancer cell specific.

Recently a study showed a longer progression free survival in patients with high levels of VEGFR2 than those with low levels in patients treated with bevacizumab and chemotherapy. This study suggests that the level of VEGFR2 are predictive of best response to antiangiogenetic therapy[16].

The durable complete response to metronomic chemotherapy in our patient highlights the importance to assess potential predictors of benefit from this treatment, and suggests further study of VEGFR2 at this regard.

This clinical case also highlights how suitable metronomic chemotherapy can be as maintenance therapy, allowing long-term treatment with no relevant toxicity.

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Conflicts of Interest: none declared

Consent: Written informed consent was obtained from the patient for publication of this case report.

Authors' contributions

ACG, LC and AR prepared the manuscript and the literature research; ET, RM, SS, PS, CD and DA corrected and revised the manuscript; LC treated and observed the patient; SB and WZ performed the histopathological and immunohistochemical examinations; DO prepared the CT scan images.

All authors read and approved of the final manuscript.

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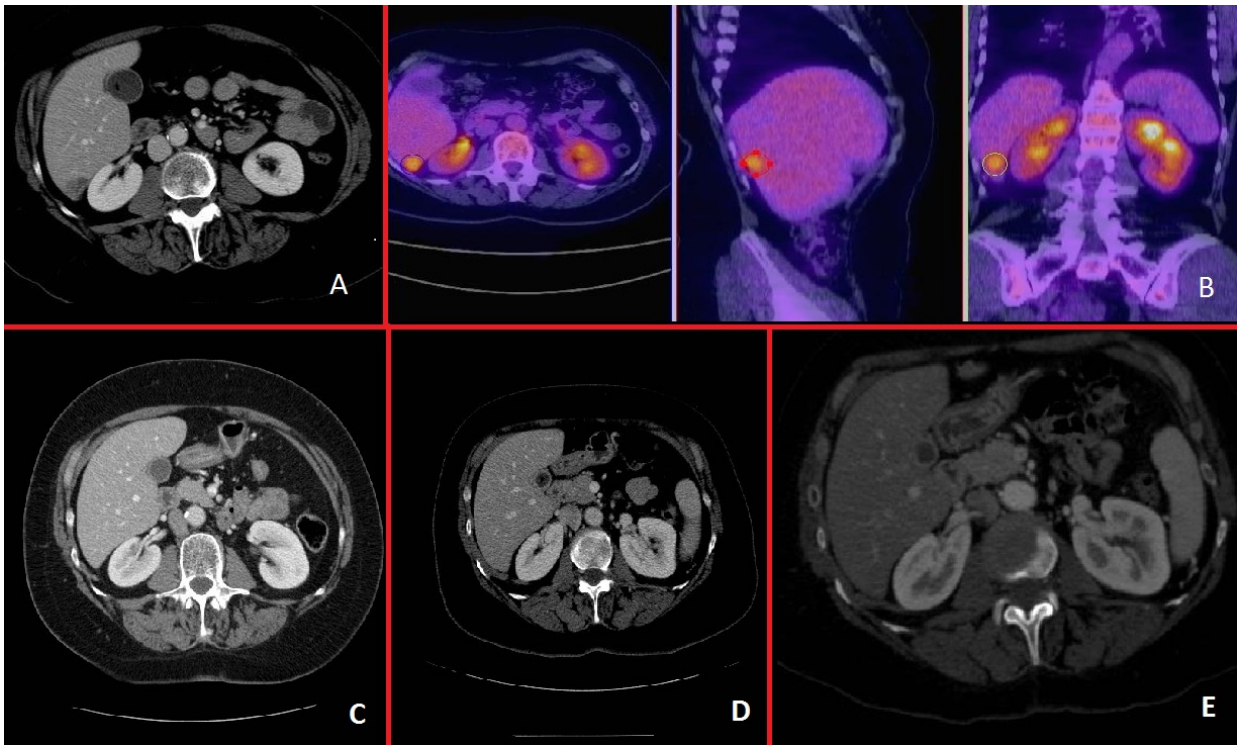
Figure legends

Fig. 1 A) CT and B) PET scans confirm presence of liver metastasis (20 mm). C) CT re-evaluation after the sixth cycle showed a partial response to treatment, with the lesion measuring 6 mm. D) CT scan performed after 6 months of maintenance therapy showed a complete response. E) CT scan after 60 months of maintenance treatment confirmed the continued complete response.

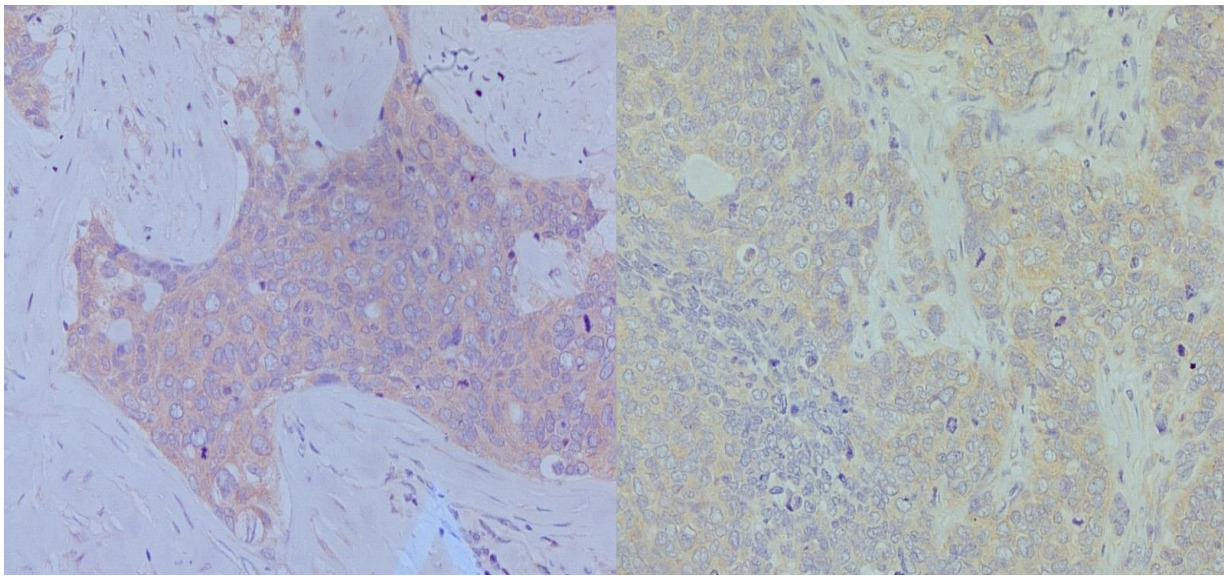
Fig. 2 VEGFR2 cytoplasmatic immunopositive tumour cells.

Figure 1



(Ingrandimento ?? x 100)

Figure 2



(Ingrandimento ?? x 100)