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Commentary

Achievements and challenges in the use of metronomics for the treatment of breast cancer

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ABSTRACT

Two interesting therapeutic proposals for cancer treatment emerged at the beginning of the 21st century. The first one was *metronomic chemotherapy*, which refers to the chronic administration of chemotherapeutic agents, in low doses, without extended drug-free periods. Then, the idea of *drug repositioning in oncology*, the use of well-known drugs that were created for other uses to be utilized in oncology, gained strength. Shortly after, the two strategies were merged in one, named *metronomics*. Both approaches share several features which make *metronomics* an appealing choice for cancer treatment: use of known and approved drugs, thus diminishing the time necessary to enter to the clinic, therapeutic effect, low toxicity, oral administration, better life quality, low costs because of the use of, generally, out of patent drugs, possibility of use, even in countries with very low economic resources.

Many chemotherapy and repurposed drugs were tested with *metronomics* approaches for the treatment of mammary cancer, the most common malignancy in women worldwide, leading to high rates of mortality. The wide range of therapeutic models studied, paralleled the wide range of responses obtained, like tumor growth and metastasis inhibition, overall survival increase, lack of toxicity, better life quality, among others. The accomplishments reached, and the challenges faced by researchers, are discussed.

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the most common cause of cancer death among women in the world. GLOBOCAN has estimated for 2018 an incidence of 24.2% and 15% of mortality caused by cancer in women worldwide [1]. According to the latest statistics, only in USA for 2019, are expected 268,600 new cases of invasive breast cancer and 41,760 deaths from breast cancer [2]. In Argentina, it represents the cancer with the highest incidence in women with a rate of 73 new cases per 100.000 inhabitants [3].

The improvement of breast cancer treatments involves surgery, chemotherapy, radiotherapy, hormone therapy, targeted therapies and, more recently, the addition of immunotherapy. Those treatments has increased the 5 years' survival rates up to 99% for localized cancer but it is still very low (27%) for metastatic disease [4]. The treatment of cancer patients at later stages is more difficult and challenging, leading both to poor clinical results and to a significant decrease in patienťs quality of life (QoL).

Standard chemotherapy treatments rely on the administration of doses of drugs near the maximum tolerated dose (MTD). They cause tumor cell death, but also, mild to severe toxicities. This kind of schedule is typically administered every 3 weeks, with a long drug-free period that allows patient's recovery from the adverse effects of chemotherapy. Unfortunately, the rest period also allows the regrowth of cancer cells, eventually also selecting more aggressive and/or resistant clones [5]. The conventional regimens have several drawbacks namely, the acquired resistance against the administered compounds, the frequent toxicities induced by them, with the consequence of QoL deterioration. Last but not least, the high costs of novel treatments are limiting the access to them in healthcare systems worldwide, and it has been a topic of discussion in many countries, not only in low and middle income, but also, in high income countries.

Those disadvantages fueled the search for effective treatments. This search entails three goals that are equally important, at least from our

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point of view, namely: i) therapeutic efficacy; ii) good QoL for the patients, an aspect somehow neglected up to now, and to which researchers are giving more importance; importantly, this goal implies the improvement of therapeutic compliance; and, not last in importance, iii) economic viability, that is to say, ensuring that all the patients have access to treatment, a situation that is not the case at present in several low and middle income countries (LMIC).

Two interesting therapeutic proposals, posing different approaches for the treatment of malignant tumors, emerged during the 21st century.

The first one was *metronomic chemotherapy* (MCT), a term coined by D. Hanahan [6] which refers to the chronic administration of chemotherapeutic agents, in low doses, without extended rest periods. The foundational papers of J. Folkman [7] and R. Kerbel [8] groups, set the bases of this treatment modality. From then on, many other researchers contributed with their investigations to increase the knowledge about mechanisms of action, therapeutic effects for different tumor types in different experimental models and, drugs suitable for their use in MCT schedules both, for individual or combined treatments. Several reviews have comprehensively summarized that work [9–16]. Nowadays, MCT is considered a valid alternative for patients who progressed to several lines of chemotherapy [17] and, importantly, for pediatric cancer in LMIC [18].

The therapeutic effect of MCT is achieved through its antiangiogenic action, mainly targeting the activated tumor blood vessels, which are permanently inhibited by the chronic administration of low doses of drugs. In contrast, standard chemotherapy, in spite of inhibiting the proliferation of tumor endothelial cells, cannot maintain this effect, because of the extended drug free brakes which allow the re-growth of tumor vasculature. MCT inhibits, not only circulating endothelial cells (CECs) but also endothelial progenitor cells (CEPs) [19,20,21], modulates pro- and/or antiangiogenic molecules like vascular endothelial growth factor (VEGF) [9,22–24], soluble VEGF receptor 2 (sVEGFR-2) [24–26], thrombospondin-1(TSP-1) [27,28], among others. Another important mechanism of action of MCT consists in the restoration of the antitumor immune response [10,29], through the inhibition of T regulatory cells [30,31] and mieloid-derived suppressor cells [32] and the stimulation of dendritic cells [33]. The inhibition of cancer stem cells (CSCs) has also been described as a probable mechanism. Kerbel *et al* found that metronomic cyclophosphamide inhibited sphere forming of C6 rat glioma CSCs. Also, it has been suggested that the inhibition of tumor vasculature disrupts CSCs, thus antiangiogenic therapy could also inhibit CSCs [34]. Also, the induction of tumor dormancy [16] or, even, a direct antitumor effect [10,35], have been proposed as responsible, at least in part, of the therapeutic effect of MCT (Fig. 1).

Several years after the beginning of MCT research, the idea of *drug repositioning in oncology*, the use of well-known and characterized drugs that were created for other uses to be utilized in oncology, gained strength [36,37]. This is an interesting approach that points to widen the repertoire of therapeutic resources without need of new pharmacologic developments but employing the ones that are already able to be used. The advance in the knowledge of relationships between chemical structure and biologic activity and in the effect of different compounds on gene expression and on metabolic and signaling pathways, made it possible to predict that many old drugs could be useful for cancer treatment. That proposal is drawing the attention of many oncologist, mainly because of the already known pharmacokinetics, pharmacodynamics and toxicities profiles, a fact that significantly diminish the time necessary to translate it to the clinic.

Shortly after, the two strategies, metronomic chemotherapy and drug repositioning, were merged in one named *metronomics* [38].

Both approaches share several features which make *metronomics* an appealing choice for cancer treatment: use of known and approved drugs, thus diminishing the time necessary to enter to the clinic, therapeutic effect, low toxicity, oral administration, better quality of life, low costs because of the use of, generally, out of patent drugs and, last in order but not in importance, possibility of use even in countries with very low economic resources.

Here, we shall summarize the main findings, both in the preclinical and the clinical setting, in the use of *metronomics* for the treatment of breast cancer.

2. Pre-clinical setting

Shortly after the launching of MCT research, with the first two published papers [7,8], different experimental models were developed in order to study the effect of drugs administered with a MCT schedule for the treatment of breast cancer.

The use of single drugs in MCT for experimental breast cancer has been scarce. Cyclophosphamide (CY), an alkylating agent that has been used for more than 60 years for standard chemotherapy treatment, can also be used in a metronomic regimen, and it was the first drug to be used as monotherapy with a metronomic schedule. The combination of metronomic daily oral low dose CY with intermittent bolus-dose CY every 3 or 6 weeks, improved the therapeutic effect, delaying tumor progression with very mild toxicity and reduction of circulating

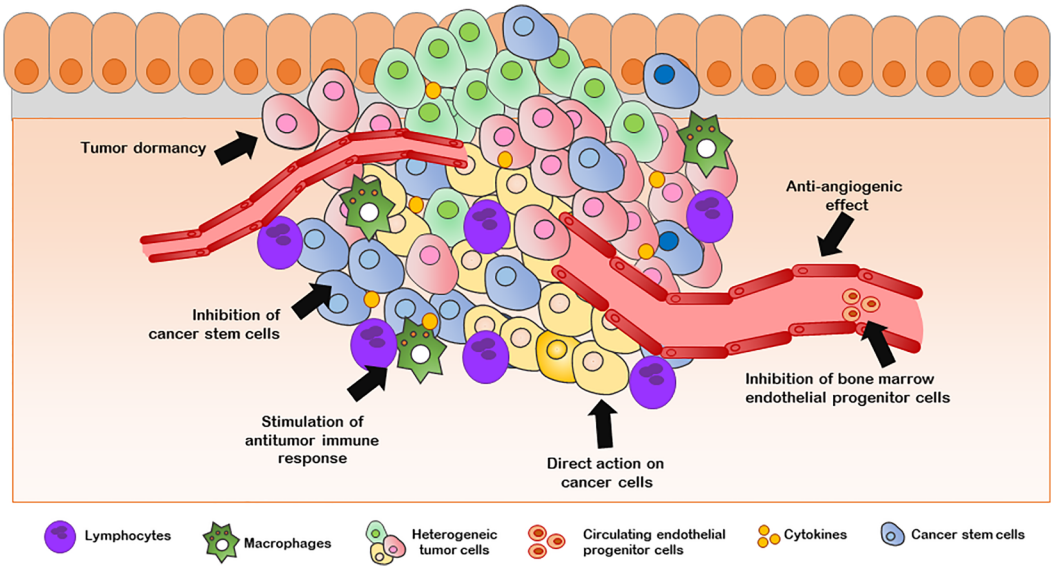

Fig. 1. Mechanisms responsible for the therapeutic effect of Metronomic Chemotherapy.

endothelial progenitor cells [39]. Moreover, MCT with Paclitaxel (PTX) showed a higher inhibition of primary and metastatic breast tumors and lower toxicity than those obtained with MTD therapy, with decrease of angiogenesis and lymphangiogenesis [40].

Apart from those experiences with single drugs, most of the researchers investigating MCT used, from the very beginning, different combinations of drugs, looking for good therapeutic results. Cyclophosphamide administered continuously in the drinking water with an antiangiogenic drug, DC101, in mice carrying tumors derived from human breast cell lines, showed a significant antitumor efficacy, proved to be safe, with a moderate grade of efficacy, and potentially applicable to chronic treatment [41]. Klement et al obtained a significant and lasting antitumor effect using a combination of low-dose/metronomic Taxol, Vinblastine, or Adriamycin with VEGFR-2-blocking antibody on multidrug-resistant tumors [42].

The effect of metronomic CY was evaluated not only as monotherapy but also in combination with many different agents. The combination of metronomic low dose CY and weekly Tirapazamine, an agent that exerts a selective cytotoxicity for hypoxic tumor cells, enhanced the antitumor effect [43]. The use of combined CY and UFT, a 4:1 M combination of Uracil and Tegafur, showed an antimetastatic effect, in the absence of effect on localized primary tumors [44]. Vascular disrupting agents (VDA) target established abnormal vasculature by binding to endothelial cells-associated tubulin. The combination of VDA OXI-4503 with a classic CY MCT showed better antitumor effect than that obtained with the mono treatments. OXI-4503 was administrated i.p. after 6 days of treatment with CY. The therapeutic effect, achieved without toxicity, consisted of diminished microvessel density and proliferation rates, together with increased tumor hypoxia and cell apoptosis [45]. Francia et al demonstrated that Trastuzumab combined with metronomic low-dose CY is effective in models of highly aggressive Her-2^{+} spontaneously metastatic human breast cancer. The authors found a prolongation in survival and minimal toxicity with the combined therapy [46]. The oral prodrug of Gemcitabine (GEM), LY2334737, instead of GEM, that is administered i.p, achieved antitumor effect alone or in combination with CY in a metronomic schedule. LY2334737 did not suppress circulating endothelial progenitor cells but increased vessel stability and functionality and caused higher blood flow [35]. We demonstrated the antitumor and antimetastatic effect of the combination of metronomic CY with Celecoxib (CEL), a cyclooxygenase 2 inhibitor, in two triple negative murine mammary tumor models [47]. Utilizing the same models, we combined MCT with CY and Doxorubicin (DOX) with similar results [48]. In both studies the therapeutic effect was achieved without toxicity. Together with a decrease of proliferation and an increase of apoptosis of the tumor cells, there was a diminution in VEGF serum levels, indicating an antiangiogenic mechanism of action.

A novel therapeutic approach was the combination of metronomic CY with USMB (ultrasound stimulated microbubbles) [49]. In this study, the combination showed significant reduced tumor growth rates and higher survival than that obtained with the individual treatments in a TNBC murine model. The therapeutic schedule combining low dose metronomic CY and Capecitabine (CAP) also produced antitumor and antimetastatic effects [50].

The appearance and use of immune checkpoint inhibitors in the treatment of several types of cancer [51] led to the assumption that combining its administration with MCT would have a synergistic effect on the antitumor immune response, increasing the therapeutic efficacy. Parra demonstrated that anti-CTLA-4 therapy together with metronomic CY, or the antibody followed by sequential therapy with GEM reduced tumor growth. However, in some cases there were relapses and the appearance of metastases [52].

Another attractive therapeutic approach is related to the use of enzyme prodrug systems, which are able to minimize systemic toxicity. The administration of CY at low doses, combined with a Tumor Vasculature-targeted Enzyme Prodrug System and Rapamycin, acted synergistically decreasing tumor volumes, increasing survival and diminishing the number of lung metastatic nodules [53].

Recently, we found that MCT with CY plus Losartan, an angiotensin II receptor antagonist, is a successful drug combination that, interestingly, caused 60% of complete regressions in a very aggressive murine model of TNBC [54]. We also shed some light into the mechanisms implicated in its antitumor effect, mainly pointing to different elements of the tumor microenvironment like angiogenic factors, immune cells and cancer associated fibroblasts, with the indirect/direct consequence of tumor cells impairment. Recently, Orecchioni studied the antitumor and antimetastatic effects of Vinorelbine (VRB), CY and 5-FU, three orally active chemotherapeutics. The drugs had effect on circulating and tumour-infiltrating immune cells in a TNBC model. The authors studied the combination of the different drugs with checkpoint inhibitors anti-PD-1 and anti-PD-L1, a kind of antibodies that are active in many types of cancers. They showed that the drugs had a synergic effect with anti-PD-L1 [55].

Doxorubicin is one of the drugs that are frequently used in the treatment of breast cancer. It was evaluated the *in vivo* antitumor effect of metronomic low-dose oral DOX combined with Chk1 (Checkpoint kinase 1) inhibitor MK-8776. The abrogation of the Chk1 checkpoint could selectively exert lethality with low-concentration DOX, against p53-deficient breast cancer by forcing the cells to accumulate Doxorubicin-induced DNA damage. The animal studies showed that MK-8776 improved the cytotoxic activity of MCT with DOX against p53-deficient cancer cells, without showing systemic toxicity [56]. Also, *in vivo* and *in vitro* assays demonstrated that Methylglyoxal, a glycolytic metabolite, produced a synergic effect in the cytotoxicity mediated by DOX and Cisplatin in MDA MB 231 and MCF cell lines. The combined treatment inhibited mammosphere formation, eliminating cancer stem cells as well as non-stem cancer cells. [57].

Other combinations of agents were studied with a MCT schedule in preclinical models, as well. Zhang and colleagues developed an effective therapeutic scheme, combining the use of CAP in a metronomic scheme with Ginsenoside Rg3, the active ingredient in ginseng extract. This combination was shown to have antiangiogenic effect, low toxicity and lower susceptibility to drug resistance [58]. Moreover, Foy studied the combination of peptide mimics with low dose chemotherapy. They obtained antitumor effect combining low-dose PTX with either HER-2 or VEGF peptide mimics. Those results were obtained with little or null cardiotoxicity [59].

The treatment that combined Sunitinib with oral Topotecan showed antitumor activity on MDA-MB-231/LM2.4 tumors, with an increase in survival. Those effects were not observed with the monotherapies [60].

Srivastava used an adjuvant scheme in a breast cancer model in which they combined anti-AnG2 antibody, anti-VEGF antibody and MCT with GEM. They obtained a significant reduction in metastatic growth [61]. The therapeutic effect of combining *Camellia sinensis* water extract and metronomic Zoledronate was investigated by Luo et al. The authors showed significant antitumor effect and proved that the combined treatment decreased lung and liver metastasis compared to the individual treatments [62].

The combination of metronomic Topotecan and Pazopanib, an antiangiogenic tyrosine kinase inhibitor, significantly enhanced antitumor activity compared to monotherapy with both drugs and prolonged survival, even in the advanced metastatic survival setting. These drugs combination showed a marked decrease in tumor vascularity and proliferative index and, also, induction of apoptosis [63].

Other researchers have explored different combinations with some interesting results. Ko and colleagues showed that the combination of metronomic Zoledronic acid plus an aqueous extract of *Coriolus versicolor*, an immunomodulatory agent, diminished tumor growth and metastasis appearance, in an intratibial breast tumor model [64].

We also studied the efficacy of a combination of Metformin and Propranolol, two repurposed drugs, in TNBC murine models. *In vitro* studies showed inhibition of proliferation, mitochondrial activity,

Table 1
Precinical investigations. Main findings with the use of metronomics.

Mice line	Tumor/Cells	Treatments [§]	Main Findings	Ref.
BALB/cJ	EMT-6/CTX	**CY**: Bolus dose 150 mg/kg every 3 or 6 weeks, plus 20 mg/kg/d in drinking water	Antitumor effect, Mild toxicity Reduction of CEPs	39
BALB/c	4 T1	**PTX**: 20 mg/kg i.p. weekly	Antitumor/Antimetastatic effects Antiangiogenic/Antilymphangiogenic activities	40
NIH Swiss CB-17 SCID mice	Human breast (435hCG17) MDA-MB-231 MDA-MB-435	**CY:** 20 and 25 mg/kg/day in drinking water plus **DC101**: 800 μg/mouse i.p. twice/week.	Antitumor effect Prolongation of survival Low toxicity	41
CB-17 SCID	MDA-MB-231 MDA-MB-435 MVB9 MD22 MPAHS TO.1. MVB9 MDA-CDDP–S4	**Group 3: VNB:** 0,5mg/kg i.p or **ADR:** 1 mg/kg i.p. or **CIS**: 1–2 mg/kg i. **Group 4: CYP-A**: 10 mg/kg, i.p. or **VER**: 20 mg/kg, i.p. every 3 days **Group 5: Group 3 plus Group 4** **Group 6: Group 3 plus DC101**: 800 μg/mouse i.p. every 3 days	Anti-tumor effect Increase in apoptosis and necrosis Minimal toxicity	42
NIH Swiss Nude	MDA-MB-231	**CY:** 20 mg/kg/d in drinking water plus **TaRZ**: 1 mg/mL i.p. weekly (25 mg/kg/wk).	Antitumor effect, Low toxicity Inhibition of cell proliferation. Antiangiogenic activity	43
CB17 SCID	MDA-MB- 231/LM2-4	**UFT**: 15 mg/kg/d plus **CY:** 20 mg/kg/d in drinking water	Antitumor/Antimetastatic effects Antiangiogenic activity Prolonged survival	44
NIH Swiss	231/LM2-4	**CY**: 20 mg/kg/day in drinking water plus **OXI-4503:** 50 mg/kg i.p. every two weeks.	Antitumor effect Diminished microvessel density Increase in apoptosis and tumor hypoxia Decrease in proliferation	45
CB17 SCID	MDA-MB-231 met2, LM2-4H2N	**CY:** 20 mg/kg in drinking water plus **TRZ**: 20 mg/kg i.p twice weekly	Antimetastatic effect Prolonged survival	46
BALB/cJ	LM2-4	**LY2334737**, 6 mg/kg/day p.o. plus **CY:** bolus, 100 mg/kg i.p. on day 1 followed by continuous 20 mg/kg/d in drinking water	Antitumor effect Increase in vessel stability, functionality and blood flow	35
BALB/c	M-234p M-406	**CY:** 30 mg/kg/day in drinking water plus **CEL:** 30 mg/kg p.o. five times/week	Antitumor/Antimetastatic effects Increase in apoptosis Decrease in proliferation and VEGF serum concentration Prolonged survival, No toxicity	47
BALB/c	M-234p M-406	**CY**: 30 mg/kg/d in drinking water plus **DOX**: 0.5 mg/kg i.p. three times/week	Prolonged survival No toxicity	48
NIH Swiss	MDA-MB-231	**CY:** 20 mg/kg/d in drinking water plus ultrasound stimulated microbubbles	Antitumor effect Sharp reduction in tumor blood flow	49
CB-17 SCID YFP-SCID	MDA-MB-231/LM2-4 MCF-7 HCI-002	**CY:** 20 mg/kg/day in drinking water, **CAP:** 100 mg/kg/day daily p.o.	Antitumor/Antimetastatic effects Decrease in MDSC and Treg cells Increase in NK and T cells	50
BALB/c	EMT-6/P, EMT-6DDP	**GEM:** bolus 160 mg/kg every 3 days, i.p., plus **CY:** 20 mg/kg/d **CY:** 20 mg/kg/d **Anti-CTLA-4:** 100 μg, day 1 and 35 μg, day 6 plus **CY:** 20 mg/kg/d **Anti-CTLA-4:** 100 μg, day 1 and 35 μg, day 6 first line and then a second-line therapy of **GEM:** 160 mg/kg, i.p every 3 days	Antitumor effect. Antiangiogenic activity	52

(*continued on next page*)

Table 1 (*continued*)

Mice line	Tumor/Cells	Treatments [§]	Main Findings	Ref.
BALB/cJ SCID	4 T1 MDA-MB-231	**TVTE**: 10 mg/kg/d i.p. plus **Selenomethionine**: 5 mg/kg/d i.p., **RAP**: 5 mg/kg/d i.p. and **CY:** 10 mg/kg/d i.p.	Antitumor/Antimetastatic effects Increase in apoptosis Decrease in proliferation and hypoxia	53
BALB/c	M-234p	**CY:** 20 mg/kg/day in drinking water plus **LOS:** 150/200 mg/kg/d in the drinking water	Antitumor/Antimetastatic effects Increase in apoptosis Decrease in proliferation, CAF and collagen production Prolonged survival, No toxicity	54
BALB/c cOlaHsd	4 T1	**CY:** 5 (low dose), 10, 20 (medium doses) and 40 (high dose) mg/kg/d, plus **VIN**: 3 (low dose), 6 and 9 (medium doses) 12 mg/kg (high dose) p.o and **5-FU**: 5 (low dose), 10–25 (medium doses), 50 mg/kg (high dose) 3 times/week i.p. for 3 weeks and **Anti-PD-L1** and **anti-PD-1**: 0.2 mg/mouse i.p. every 2 days for a total of 5 doses.	Antitumor/Antimetastatic effects Modifications of circulating and tumor-infiltrating immune cells.	55
BALB/c nude	MDA-MB-231 MCF-7 HCC1937 HCC1954	**DOX/DOCA:** 1.25, 2.5, or 5 mg/kg p.o. once a day, plus **MK-8776**: 30 mg/kg, i.p. once a week.	Antitumor effect Increase in apoptosis Low toxicity	56
Swiss Albino	MDA MB 231 MCF 7 line EAC	**Group DoxMG**: **DOX** 3 mg/kg plus **MG** 10 mg/kg 5 days **Group CisMG: CIS** 3 mg/kg once a week plus MG 10 mg/kg-5 days	Antitumor effect	57
BALB/c	4 T1 cell	**CAP:** 200 mg/kg o.p. plus **Ginsenoside Rg3**:10 mg/kg/d p.o.	Antitumor effect, No weight loss Microvessels decrease Low tumor VEGF expression	58
BALB/c	TUBO cells	**PTX**: 60 μg plus **: HER-2 and VEGF peptide mimics**: 300 μg	Antitumor effect Decrease in proliferation and microvascular density	59
SCID	MDA-MB-231/LM2.4	**Sunitinib**: 60 mg/kg p.o. plus **TOP:** 1 mg/kg/d p. o.	Antitumor effect. Prolonged survival	60
SCID	4 T1-luc	**Ang2 Ab**: 6 mg/kg, i.p, or **Ab VEGF**: 10 mg/kg i.p. twice a week	Antitumor/Antimetastatic effects Reduced vascularization. Inhibition of recruitment of bone marrow-derived myeloid cells	61
BALB/c	4 T1	***Camellia sinensis*:** 0.6 g/kg/d orally fed every day plus **ZOL:** 0.0125 mg/kg, i.p. twice a week	Antitumor/Antimetastatic effects Increase in tumor cell death. Anti-osteolytic effect No toxicity	62
SCID	MDA-MB-231, MDA-231/LM2-4	**TOP:** 1 mg/kg/d p.o. plus **Pazopanib:** 150 mg/kg /d p.o. and **Sunitinib**: 60 mg/kg/d p.o.	Antitumor/Antimetastatic effects Decrease in proliferation Increase in apoptosis and microvascular density Inhibition of HIF1α and ABCG2 Prolonged survival	63
BALB/c nu/nu nude	MDA-MB-231-TXSA	**CV:** 1 g/kg, orally fed daily plus **ZOL**: 0.0125 mg/kg, i.p. twice a week.	Antitumor/Antimetastatic effects Decrease in CD34 and MMP-2 expression	64
Balb/c CBi	4 T1 M-406	**MET:** 400 mg/kg/d in drinking water plus **PROP** :7 mg/kg/d in drinking water	Antitumor/Antimetastatic effects Increase in apoptosis Decrease in proliferation No toxicity	65

§: not all the experimental group are mentioned; **5-FU: 5**- Fluoruracil; **ADR**: Adriamycin; **Ang2 Ab:** anti-angiopoietin-2; **CAF**: tumor associated fibroblasts; **CAP**: Capecitabine; **CEL:** Celecoxib; **CIS:** Cisplatin; **CTLA-4:** cytotoxic T-lymphocyte–associated antigen 4**; CV:** *Coriolus versicolor*; **CY**: Cyclophosphamide; CYP-A**: Cyclosporin A; DC101**: monoclonal antibody for mouse VEGFR-2 (vascular endothelial growth factor receptor 2); **DOX**: Doxorubicin, **DOX/DOCA:** complex of DOX plus deoxycholic acid**; GEM:** Gemcitabine; **LOS**: Losartan**; LY2334737:** oral prodrug of GEM**; MDSC**: myeloid-derived suppressor cells; **MET**: Metformin; **MG**: Methylglyoxal; **MK-8776:** Chk1 (Checkpoint kinase 1) inhibitor; **NK:** Natural Killer cells; **OXI-4503:** vascular disrupting agent**; PD-1**: programmed cell death-1; **PD-L1**: programmed cell death-1 ligand; **PROP**: Propranolol; **PTX**: Paclitaxel; **RAP:** Rapamycin; **TOP:** Topotecan**; TRaZ:** Tirapazamine; **Treg**: regulatory T cells; **TRZ**: Trastuzumab; **TVTE**: Tumor Vasculature–Targeted Enzyme; **UFT**: 4:1 M combination of uracil and tegafur; **VEGF**: vascular endothelial growth factor**; VER:** Verapamil; **VIN:** Vinorelbine; **VNB**: Vinblastine; **ZOL:** Zoledronate.

Table 2
Most relevants clinical trials of metronomics in breast cancer patients.

Phase (n)	Treatment Scheme	Endpoints	Outcomes							Ref.
			CR	**PR**	**SD**	**RR**	**CB**	**TTP (mo)**	**OS (mo)**	**Other**	
NEOADYUVANT SETTING											
II (114)	**Arm A**: LET (n = 57)	RR	23/57	18/57	12/57	72%	–	–	–	Toxicity was generally mild	66
	Arm B: LET + CY (n = 57)		25/57	25/57	4/57	88%	–	–	–		
II (20)	Arm Her 2+: weekly PTX and DOX + CY + TZB (n = 9) Arm Her 2-: weekly PTX and DOX + CY (n = 11)	Safety and RR	5/9 2/11							Treatment was discontinued because toxicity related issues	69
II (29)	PLD + CY + Surgery after 8 cycles of PLD + RT	RR, TL	0/29	18/29	10	62%	–	–	–	BCS: 45%; MAST: 45%; Bilateral surgery: 10%. Good tolerability, minimal G3-4 toxicities.	67
II (34)	ECF + PTX + CY	RR, Ki67 decrease	6/34	25/34	3/34	91%	–	–	–	High pCR rates, reduction of Ki-67, and low toxicity profile	68
METASTATIC SETTING											
II (63)	CY + MTX	RR, TL	2/63	10/63	8/63	19%	32%	2.8	–	Minimal GI or II HAE	22
II (178)	*Arm A*: CY + MTX (n = 90)	PFS, OS	3/86	15/86	18/86	21%	42%	3.8	18.2	Well tolerated, toxicity was generally mild	23
	Arm B: CY + MTX + TH (N = 88)		3/85	7/85	25/85	12%		4.1	17.1		
II (42)	CY + MTX	RR, Toxicity	0	7/42	6/42	17%§	31%	–	–	PFS: 7 ± 0.18 mo. HAE and NHAE were minimal	71
N/A (28)	CY + MTX	N/A	1/28	13/28	8/28	50%§	61%	3.7		One-year survival: 4 (28.6%)	70
I/II (41)	CY + MTX + D + PRED	CB, TTP, OS	1/41	6/41	3/41	17%	24%	2.5	12	16 patients with non-prior chemotherapy for MBC; no significant differences in RR or CB. Toxicities were minimal	77
N/A (61)	Arm A: CY (n = 22)	RR, TTP, Safety	0	3/22	9/22	18%§	55%	4.2	13.8	Non statistical differences between cohorts. HAE were extremely infrequent	72
	Arm B: CY + MTX (n = 39)		0	8/39	12/39		51%				
CCR (33)	*Arm A*: F, after PD: F + CY + MTX (n = 20) *Arm B*: F + CY + MTX from the start (n = 13)	N/A	0	1/33	17/33	3%§	56%	–	–	Whether the treatment combination was concomitant or sequential, PFS and OS did not significantly differ. The regimen was well-tolerated	76
I (23)	CY + MTX + VAN	Safety and RR	0	2/20	3/20	10%§	25%§	–	–	Maximum VAN dose: 200 mg with modest clinical activity and mild toxicity	75
N/A (22)	TZB + CY + MTX	N/A	0	4/22	6/22	18%	46%	6	–	Clinical toxicity was generally mild. G ≥ 2 reversible liver toxicity and leukopenia.	71
I (21)	CY + MTX + 1E10-Alum vaccine	Safety, RR	2/21	3/21	8/21			9.82	12.93	Most AE were reported and most of them were classified as G1	78
II (46)	CAP + CY + BEV	CB, Toxicity	1/46	21/46	19/46	48%	68%§	10.5		Toxicity was generally mild	83
I (36)	VRB + CAP	DLT	2/32	10/32	–	37%§	–	5.6	25.9	HAE and NHAE were mild	85
II (58)	CAP	CB, TTP, OS, Toxicity.	3/58	11/58	36/58	24%	62%§	7	17	Treatment was well tolerated. HAE were infrequent and mild	79
II (68)	CAP + CY	TTP, RR, OS, Safety	1/66	19/66	–	30%	53%	5.2	16.	Treatment was well tolerated.	81
II (60)	CAP + CY	RR, Toxicity, TTP, OS	1/60	12/60	21/60	22%	57%	7	16	For RespP OS: 24 ± 10.01 mo. Treatment was well tolerated	80
II (47)	DTX + CAP + CEL	CB, TTP, OS, Toxicity	–	13/38	–	34%§	42%	3.3	9.8	Treatment was well tolerated. Most AE were diarrhea, plantar-palmar erythrodysesthesia.	87
II (24)	CAP + CY + ERL + BEV	Safety, RR, OS, PFS, CB	1/24	14/25	–	62%§	75%§	10.7	108	Toxicity was generally mild	21
I/II (34)	VRB + CAP	DLT	5/31	9/31		16%	58%	–	–	Treatment was feasible and well tolerated during prolonged periods.	86
II (41)	F + CAP	PFS, TTP, RR	2/41	8/41	28/41	24%§	59%§	26.94	28.65	Palmar plantar erythrodysesthesia was the most common cause of dose reductions	82
CCR (52)	PLD	CB, TTP, OS	–	8/45	17/45	18%	45%	4.2	18.35	Treatment was well tolerated, AE were minimal	93
II (34)	VRB	RR, PFS, OS, Safety	2/34	11/34	10/34	38%	68%	–	15.9	Toxicity was generally mild	92
II (29)	CY + MA	RR, Safety	7/29	2/29	3/29	31%	41%	7.4	13.4	The treatment was active and well tolerated.	91
II (36)	WBRT + TMZ + VRB 70 mg/m^2	RR, PFS, OS, Safety.	3/36	16/36	9/36	52%	77%	–	11	All AE were low and manageable	94
N/A (157)	DTX + TZB (Her2 +)	RR	7/157	54/157	52/157	38%	72%	8.8	27.7	3-year OS: 39%. The regimen was well-tolerated	89

(*continued on next page*)

Table 2 (*continued*)

Phase (n)	Treatment Scheme	Endpoints	Outcomes							Ref.
			CR	PR	SD	RR	CB	TTP (mo)	OS (mo)	Other	
II (15)	CY + CEL	CB, TTP, PFS, OS, Safety	–	1/15	6/15	7%	46.7%	3.5	11	1-year OS: 46.7%. No serious HAE or NHAE were reported	24
N/A (84)	NPLD + 5-FU + VCR + CY + PRED + TZB (HER2 +)	RR, CB, PFS, OS	1/84	37/84	23/84	45%	73%	–	21	There were no G4 AE. 3 patients experienced an asymptomatic LVEF.	90
MAINTENANCE SETTING											
III (1 5 8)	Arm 1: FEC-100 + DTX + carboplatin + metronomic CY + MTX (n = 78) Arm 2: EFC + DTX + carboplatin (n = 80)	DFS and OS	–	–	–	–	–	28 24	37 29	Mild toxicity, significant improvement in survival.	98

§ calculated with data given by authors; **5-FU**: 5-Fluoruracil; **AE**: hematological adverse events; **BEV**: Bevacizumab; **CAP**:Capecitabine; **CB:** clinical benefit; **CEL**: Celecoxib; **CR:** complete response; **CY:** Cyclophosphamide; **D**: Deltaparine; **DLT**: dose-limiting toxicity; **DOX**: Doxorubicin; **DTX**: Docetaxel; **ECF**: Epirubicin, Cisplatin, 5-Fluorouracil; **ERL**: Erlotinib; **F**: Fulvestrant; **FEC-100**: 5-Fluorouracil, Epirubicin, Cyclophosphamide; **LET**: Letrozole; **MA**: Megestrol acetate; **MTD**: maximum tolerated dose; **MTX**: Metotrexate; **N/A:** not available; **NHAE**: non-hematological adverse events; **N-PLD**: Non Pegylated Liposomal Doxorubicin; **OS (mo):** overall survival (months); **PLD**: Pegylated Liposomal Doxorubicin; **PFS**: progression free survival; **PR:** partial response; **PRED**: Prednisone; **PTX**: Paclitaxel; **ResP**: responder patients; **RR**: response rate; **RT**: Radiotherapy; **SD**: stable disease; **TTP (mo)**: time to progression (months); **TZB**: Trastuzumab; **VAN**: Vandetanib; **VCR**: Vincristine; **VRB**: Vinorelbine; **WBRT**: Whole Brain Radiotherapy.

migration and invasion. The drugs combination, tested *in vivo*, significantly decreased tumor growth and prevented metastasis in two TNBC murine models [65].

The main characteristics of the mentioned investigations are summarized in Table 1.

The above mentioned preclinical studies gave evidence of therapeutic effect together with low toxicity profiles of MCT for breast cancer treatment and, in several cases, paved the way to their clinical evaluation.

3. Clinical setting

Since its conception, several trials have been published about the activity and safety of metronomic chemotherapy in the treatment of breast cancer. Most of the patients included in those clinical trials had advanced, multiple metastasized, heavily pretreated tumors, which developed progressive disease after standard chemotherapy. However, there are also some evidence of activity in locally advanced and neoadjuvant settings. The most common chemotherapy agents used in those metronomic schemes were CY, Methotrexate (MTX) and CAP, accompanied, or not, with some other hormonal or biological targeted agents.

3.1. Metronomic chemotherapy in the neoadjuvant setting of locally advanced breast cancer (LABC) treatment

It is well known that after several lines of chemotherapy, the chances of response decrease significantly line after line. There are not many trials using MCT in the neoadyuvant setting for women with LABC. The gold standard for patients with LABC with positive hormone receptors are the antagonists of the estrogen receptor, like Tamoxifen, or the non-steroidal aromatase inhibitor Letrozole (LET). This is also the case for elderly patients, when chemotherapy is not allowed. A randomized phase II trial conducted by Bottini et al. [66] using aromatase inhibitor LET and LET plus metronomic CY, 50 mg p.o. daily, showed a response rate of 88%; unfortunately, the study was not designed to compare response between arms, and both (LET and LET + CY) demonstrated to be effective. However, the comparison of both arms suggested that the addition of CY increased the activity of LET, explaining the higher response rate in that arm. Two pathological complete responses (CR) were observed in both arms, but the addition of metronomic CY did not seem to increase the CR rate. Dellapasqua [67] designed a Phase II trial to evaluate safety and activity of Pegilated Liposomal Doxorrubicin (PLD) plus metronomic CY in patients who were not suitable to receive a standard chemotherapeutic treatment due to age or co-morbidities. The response rate (RR) was 62.1%, but no complete responses were observed. The treatment was well tolerated but, unfortunately, showed a limited activity in the neo-adjuvant setting. Cancello et al, showed that metronomic CY plus weekly PTX after epidoxorubicin-cisplatin-fuorouracil (ECF) as neoadjuvant treatment decreased Ki-67 and a high proportion of patients had complete pathological response [68]. A small trial examined the feasibility of neoadjuvant metronomic chemotherapy in two cohorts, HER2+ and HER2- locally advanced BC patients; this trial was negative and it was discontinued due toxicity related to treatment [69]. In Table 2 are summarized the main findings of the cited clinical trials.

3.2. Metronomic chemotherapy in the metastatic setting of breast cancer treatment

3.2.1. Metronomic Cyclophosphamide plus Methotrexate and other agents in metastatic breast cancer

As previously said, CY is one of the most widely investigated drug in different metronomic schemes. The metronomic dose used in several clinical trials has been set up empirically in 50 mg p.o. daily [22]. Indeed, the combination of CY + MTX (Methotrexate), was the first

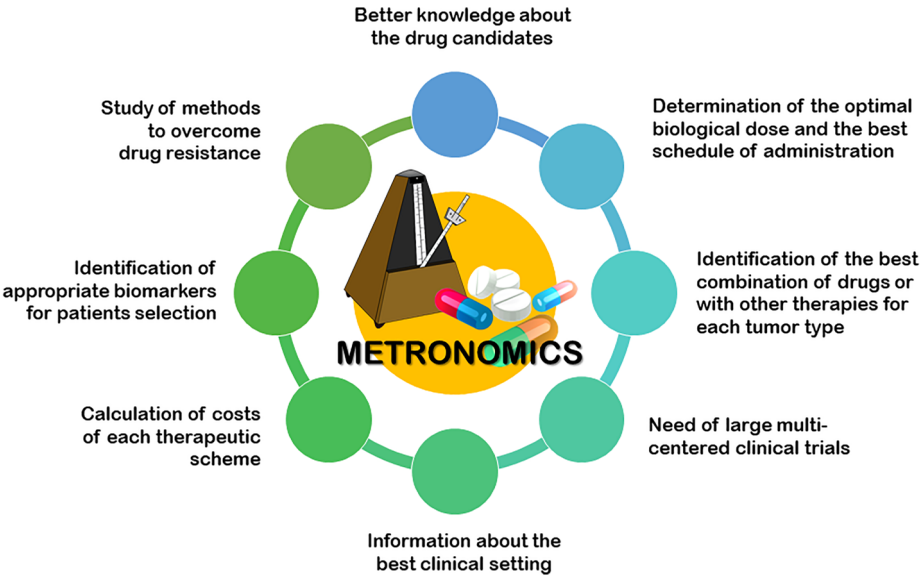

Fig. 2. Future challenges in the field of Metronomics.

metronomic chemotherapy protocol reported in the clinic in breast cancer patients by Colleoni [22] in 2002; their results stimulated the development of subsequent studies by others. They found a response ratio of near 20% with low and manageable toxicity. The addition of Thalidomide (TH) to the metronomic protocol, did not improve the RR [23] in another study conducted by the same author. A RR of 50% and clinical benefit of 61% were obtained by Soriano-García et al. in a small clinical trial [70]; moreover, Salem reported encouraging RR and CB (17% and 31%, respectively), in salvage therapy context [71]. On the contrary, Gebbia et al. studying the effect of adding MTX to metronomic CY, did not find statistically significant differences between cohorts [72]. The addition of biological agents like Trastuzumab (TZB) or Bevacizumab (BEV) might also add a synergic antiangiogenic effect in HER2-positive patients, leading to a higher CB [73,74]. Many others tried to get similar results in small trials using this scheme alone or combined with other anti-cancer agents like the VEGFR inhibitor Vandetanib (VAN) [75], Fulvestrant [76], non-anticancer drugs like TH [23], Dalteparin and Prednisone [77] or Idiotype vaccine [78], however outcomes showed non-significant therapeutic improvements.

3.2.2. Metronomic Capecitabine, and derivatives, in metastatic breast cancer

Oral CAP allows the treatment of breast cancer, simulating the effect of continuous infusion of 5-FU, with the advantage of its oral administration. The efficacy of CAP as monotherapy has been well established for metastatic breast cancer resistant to both, taxanes and anthracyclines. The metronomic dose of CAP has not been established, and ranges from 1000 to 1500 mg (500 mg p.o. three times/day), depending if it is given alone or combined with other agents. Metronomic CAP, as single agent therapy, demonstrated to be very effective with a RR of 24% in heavily pretreated MBC [79]. These outcomes vary from 21% to 30% with the addition of CY [80,81] or Fulvestrant [82]. Interestingly, the addition of CY and BEV allowed obtaining an even better RR of 48% and CB of 68% [83]. A Phase II Trial conducted by Montagna and colleagues, designed to study the safety and the RR of a 4 drug combination including CAP + CY + ERL + BEV [21] reached a RR of 62% and 75% of CB, with moderate toxicity. In spite of these results, the addition of biological agents, like BEV, to standard metronomic CAP is still controversial and do not provide enough benefit to patients that justify its cost [84].

Other agents like VRB + CAP, demonstrated activity [85,86]. Also, Docetaxel (DTX) has been added to CAP in patients with prior anthracycline exposure; metastatic breast cancer patients showed a RR of 34% and a CB of 42% [87] with this metronomic treatment. Other prodrugs from the family of Fluoropyrimidine, like TS-1, showed a RR of 47% and a high disease control rate (CB) of 97% in combination with Irinotecan [88].

3.2.3. Other schemes of metronomic treatment in advanced breast cancer

Many other schemes of MC have been tested. They include DTX [89]; a 5-drug combination involving non-pegylated liposomal doxorubicin (NPLD) [90]; CY + MA (Megestrol acetate) [91]; VRB [92]; CY + CEL [24] ; and PLD [93]. Interestingly, Addeo et al. found a 52% RR and 77% CB in the treatment of brain metastases with concurrent low dose Temozolomide (TMZ) + metronomic oral VRB, and whole-brain radiotherapy in women with advanced breast cancer with previously untreated brain metastasis [94]. The highest OS was obtained in a large trial using DTX + TZM (27.7 months) [89], followed by the scheme with a 5-drug combination (21 months) [90] and PLD (18.35 months) [93]. Several authors described the activity of empirical low doses of oral etoposide in breast cancer [95]. In a phase II trial it was demonstrated the activity of chronic etoposide in MBC with an ORR of 35% and PR of 21% [96]. Also, the most recent multicenter phase II trial of oral etoposide showed a CB of 21.3% with a median PFS of 4.5 months [97].

Some metronomic schemes have also been evaluated as maintenance therapy in TNBC patients. Nasr KE et al developed a large clinical trial using maintenance metronomic after adjuvant FEC (Fluorouracil-Epirubicin-CY) and DTX and a control group without maintenance; the study arm showed that DFS and OS were 28 and 37 months, respectively, compared to that of control groups of 24 and 29 months [98]. The treatment schemes, endpoints and main outcomes of the cited clinical assays are shown in Table 2.

4. Challenges and future directions

In order to establish this proposal as a therapeutic tool among those included in the standard of care for breast cancer, several issues are yet to be solved. The experience obtained up to now, with advanced breast cancer patients, allow us to conclude that MCT is an active and safe therapeutic modality but, in many cases, still empirical. Having the answers to those issues will enable to design optimal metronomic chemotherapy schemes with improved outcomes (Fig. 2).

We should know the best drug candidates, a quality that implies not

only clinical effectiveness but also low toxicity, possibility of oral administration, known mechanisms of action and low cost. Also, there is the need to identify the best drug, or drugs combination, or drugs combined with other targeted or non-targeted agents, for each tumor or histologic type.

Another important issue to be solved is the determination of the optimal biological dose (OBD) for each agent and the intervals of administration needed to reach it. Shaked [99], working with preclinical tumor models, proposed the use of CEPs as biomarker to determine the OBD in MCT treatments. Bocci and Kerbel called the attention on the lack of well-established pharmacokinetic profiles and considered it the main obstacle for proposing MCT treatments [100].

It is also necessary to have data about the best clinical setting, be it neo-adjuvant, adjuvant or maintenance, for using *metronomics* in each clinical case. Moreover, we need to establish the suitability of interpolating this therapeutic modality with other treatment approaches, for which different clinical trials should be proposed and developed. For instance, one possibility, among several, could be reducing the tumor mass with standard chemotherapy and then continuing with metronomics.

Although the generation of resistance to MCT was initially thought to be significantly lower than that generated by MTD because of the different cell target, MCT also induce resistance, albeit by different mechanisms [101]. Different strategies to solve this problem were studied in preclinical and clinical models using thrombospondin 1 peptide ABT-510, VEGF pathway inhibitors, AKT inhibitor V, autophagy inducer rapamycin, autophagy inhibitor hydroxychloroquine and the tumor stroma modulating agents rofecoxib and pioglitazone. These therapeutic combinations may represent promising approaches to amplify MCT efficacy [102]. Hence, much knowledge is needed about which drugs combination/schemes induce less resistance.

Another important issue to be addressed is the patient selection. The identification of biomarkers, particularly the predictive ones, which can significantly point out the patients that will respond to the treatment, is still very scarce. Linked to this subject it is the method by which, the efficacy of the therapy that determines when to stop MCT, is assessed. Indeed, response criteria should be reviewed, because the Response Evaluation Criteria in Solid Tumors (RESIST) or WHO criteria, which are designed to detect early effects of cytotoxic chemotherapy, may not provide a complete assessment of response in metronomic based therapies [103]. Also, the use of *metronomics* in those scenarios of bad performance status, increases the chances of not having good therapeutic results.

Phase III trials for breast cancer treatment with *metronomics* must be developed, in order to solve many of the unanswered questions. There are scarce examples of Phase III clinical protocols [104,105]. Consequently, based in the previous considerations, large multi-centered clinical studies are needed to validate the best selection of the *metronomics* treatment.

Another important need is to increase the information about the benefits of the metronomic therapeutic approach in specialized media, changing from traditional to modern communication media. Most of the time it is mainly used for palliative care, even when the evidence showed that is actually active.

Last but not least, it is relevant to know in detail the costs of each therapeutic scheme, in order to choose those suitable for each country situation. As it is already known, standard chemotherapy implies high expenses, not only because of the costs of the patented drugs administered, but also due to the need of intravenous administration, which implies hospitalization, specialized personnel and disposable items. Also, the costs of counteracting the adverse effects of the treatments should be added. On the other hand, MCT generally uses generic drugs in oral formulations, with low toxicity, thus avoiding all the related expenses. Therefore, the convenience of its use in countries with limited medical resources is crystal clear. Moreover, André and colleagues [38] proposed the combination of metronomics with new business models in order to make easier and more affordable and efficient the treatment of cancer in LMIC and, simultaneously, to favor the research in this area. So, further efforts should be done to fulfill those needs.

Ten international experts, in a report describing the current status of the treatment of advanced breast cancer with MCT, concluded that the already published preclinical and clinical data "is robust enough to recommend considering this kind of therapy as a treatment option in patients with MBC" [106]. In our opinion we should follow this recommendation and, at the same time, continue developing the clinical protocols that will provide all the lacking information about how to obtain the best results with the use of *metronomics*.

Credit authorship contribution statement

O. Graciela Scharovsky: Conceptualization, Writing - review & editing. **María José Rico:** Writing - original draft. **Leandro E. Mainetti:** Writing - original draft. **Herman A. Perroud:** Writing - original draft. **Viviana R. Rozados:** Conceptualization, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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