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Metronomic Chemotherapy in Pediatric Oncology: A Way Forward for Low-income Countries?

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A. Abstract

Over the last decade, metronomic chemotherapy (MC)—the chronic administration of chemotherapy at relatively low, minimally toxic doses on a frequent schedule of administration, at close regular intervals, with no prolonged drug-free breaks—has emerged as a potential strategy to control advanced/refractory cancer disease. It was originally believed to work primarily through anti-angiogenic mechanisms, but recently, other mechanisms of action have been reported. MC has the property to kill resistant cancer cells and/or to hamper tumor growth while significantly reducing unwanted toxic side effects.

Here, we will expose preclinical data about MC and will briefly review the data regarding clinical experience with this kind of anti-cancer treatment in children. Based on these data, we will foresee potential new developments in MC in pediatric oncology, with an emphasis on countries with limited resources.

B. Introduction

Most conventional anti-cancer chemotherapeutic strategies are given to kill as many tumor cells as possible. This can be achieved by using combinations of anti-cancer agents that are administered at doses close to maximum tolerated dose (MTD). Indeed, the tolerance of chemotherapy is very frequently a limiting factor that prevents further increases in the doses of chemotherapy regimens so that limiting toxicities lead to the definition of MTD. The administration at MTD will also limit the frequency at which chemotherapy can be administered, usually leading to chemotherapy administration every 2–4 weeks. The time in between is mandatory for the non-malignant tissues to recover from the toxic effects of chemotherapy. Meanwhile, remaining tumor cells may take advantage of the chemotherapy breaks to resume cellular proliferation. This strategy is notable because it may also contribute to the

emergence of tumor resistance and in turn, tumor progression. Many mechanisms contribute to the development of resistance to chemotherapy[1]. As of today, many patients with cancer still cannot be cured using current chemotherapeutic approaches that mostly rely on the systemic administration of cytotoxic drugs at MTD [2], so new strategies are urgently needed.

In the last decade, angiogenesis has progressively gained a central position in the battle against cancer and has led to the development of new therapeutic tools[3,4]. Indeed, initially anticipated as a possible target by Folkman[5], angiogenesis was also proposed to be an indirect target that could be a mechanism to overcome cancer cell resistance[6]. Anti-angiogenic agents that target vascular endothelial growth factor (VEGF) or one of its receptors Flt1, KDR, or Flt-4 (VEGFR1-3) have now become a part of standard treatment for several malignancies in adults[7,8], although successes in pediatric oncology remain rare[9,10].

Among the anti-angiogenic approaches, metronomic chemotherapy (MC) is defined as the frequent administration of chemotherapy drugs at doses below the single maximal tolerated dose (MTD) and without prolonged drug-free breaks[11,12]. MC has been reported to significantly reduce adverse events (AEs) usually associated with chemotherapy. Although clinical data about MC in pediatric oncology remains sparse[11, 12], this approach may be well suited to and represents a genuine alternative solution for children with poor prognosis or refractory disease, potentially as a maintenance therapy following multimodal treatment[13]. Furthermore, its low cost and limited toxicity make MC a very attractive therapeutic option in low- and middle-income countries[14, 15].

The aim of this article is not to provide an exhaustive review about the preclinical and clinical data about MC but to propose new ideas to foresee its potential development in countries with limited resource based on previously published data.

B. References

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C. A Brief History of Metronomic Chemotherapy

Slightly more than 10 years ago, the chronic administration of low- and minimally toxic doses of chemotherapy (MC) was reported to be an efficient alternative to MTD chemotherapy[1,2]. Bowder et al.[2] demonstrated that cyclophosphamide, when administered at doses lower than the MTD with shorter intervals and without extended rest periods, could have an anti-angiogenic effect resulting in tumor control for an extended period.

Furthermore, this approach led to better results than those obtained with the MTD schedule when treating drug-sensitive Lewis lung carcinoma and L1210 leukemia[2]. Remarkably, similar findings were obtained with cyclophosphamide-resistant tumor cell lines (Lewis lung carcinoma cell line and murine mammary carcinoma cell line, EMT-6), for which metronomically administered cyclophosphamide restored tumor sensitivity. This schedule of cyclophosphamide together with the anti-angiogenic agent targeting VEGFR2 (TNP-470) amongst others was even more efficient than low-dose cyclophosphamide alone in obtaining tumor growth inhibition in vivo. Meanwhile, toxicity was reduced compared to chemotherapy used at MTD[2]. Klement et al. reported the effect of low-dose continuous chemotherapy as a possible anti-angiogenic strategy in a mice model of neuroblastoma[1]. Using a dose of vinblastine that did not display any anti-proliferative effect on 2 neuroblastoma cell lines in vitro but a marked anti-proliferative effect on endothelial cells, they showed tumor growth inhibition in nude mice bearing tumor xenografts from both the neuroblastoma cell lines. Interestingly, by using low doses of vinblastine together with a monoclonal antibody blocking VEGFR2 (DC101), long-term tumor regression was observed. Toxicity associated with the use of MTD chemotherapy in mice was not seen in animals treated with the metronomic approach. In both studies[1,2], an anti-angiogenic effect was demonstrated to be associated with the anticancer effect.

Following these 2 reports, Hanaghan[3] coined the term “metronomic” to the concept of “anti-angiogenic low-dose chemotherapy,” and Fidler[4] proposed that cancer should be regarded as a chronic disease and treated as a chronic disease and therefore that, trying to control it with a long-term perspective may be more realistic and successful than trying to eradicate it at all cost. At the time, MC was even envisioned by some oncologists as the future of chemotherapy [5].

Many preclinical data confirmed the initial findings[5-8]. Then, the first clinical trial investigating the role of MC in women with breast cancer was reported by Colleoni et al. in 2002[9]. In this report, 64 patients with metastatic breast cancers were treated with a combination of low-dose methotrexate and cyclophosphamide leading to a clinical benefit lasting more than 6 months in 32% of the patients[9]. Detailed results of subsequent clinical trials using different drugs, drug combinations, schedule of administration, doses, and tumor types, which were undertaken in adults and children have been intensively reviewed elsewhere [10]. Favorable results have been obtained in patients with, for instance, ovarian cancer[11], prostate cancer[12], and non-Hodgkin lymphoma[13]. MC seems to be very promising in patients with breast cancer[14]. MC studies have also showed that several anticancer agents can be used for this application due to the very high sensitivity of endothelial cells to chemotherapy. Thus, various drugs have been used for metronomic applications; these include anti-tubulin agents such as vincristine, vinblastine, vinorelbine, or taxanes[15]; cyclophosphamide; etoposide; and temozolomide (TMZ)[6, 10].

Of note, metronomic scheduling had already been used for decades in a palliative setting before the discovery of its anti-angiogenic potential with, for instance, the use of oral etoposide[16]. Moreover, maintenance therapy as used in acute lymphocytic leukemia, with oral weekly methotrexate and daily 6-mercaptopurine is also a long-lasting example of the successful application of MC. Despite a growing interest, reflected by an increasing number of publications dedicated to MC[17], it remains nevertheless marginal when compared to MTD chemotherapy. Inconsistently, many oncologists have adopted the metronomic approach for many targeted agents such as mammalian target of rapamycin (mTOR) inhibitors, Gleevec®, and Sutent® but paradoxically, have never used them in the MTD way[18].

C. References

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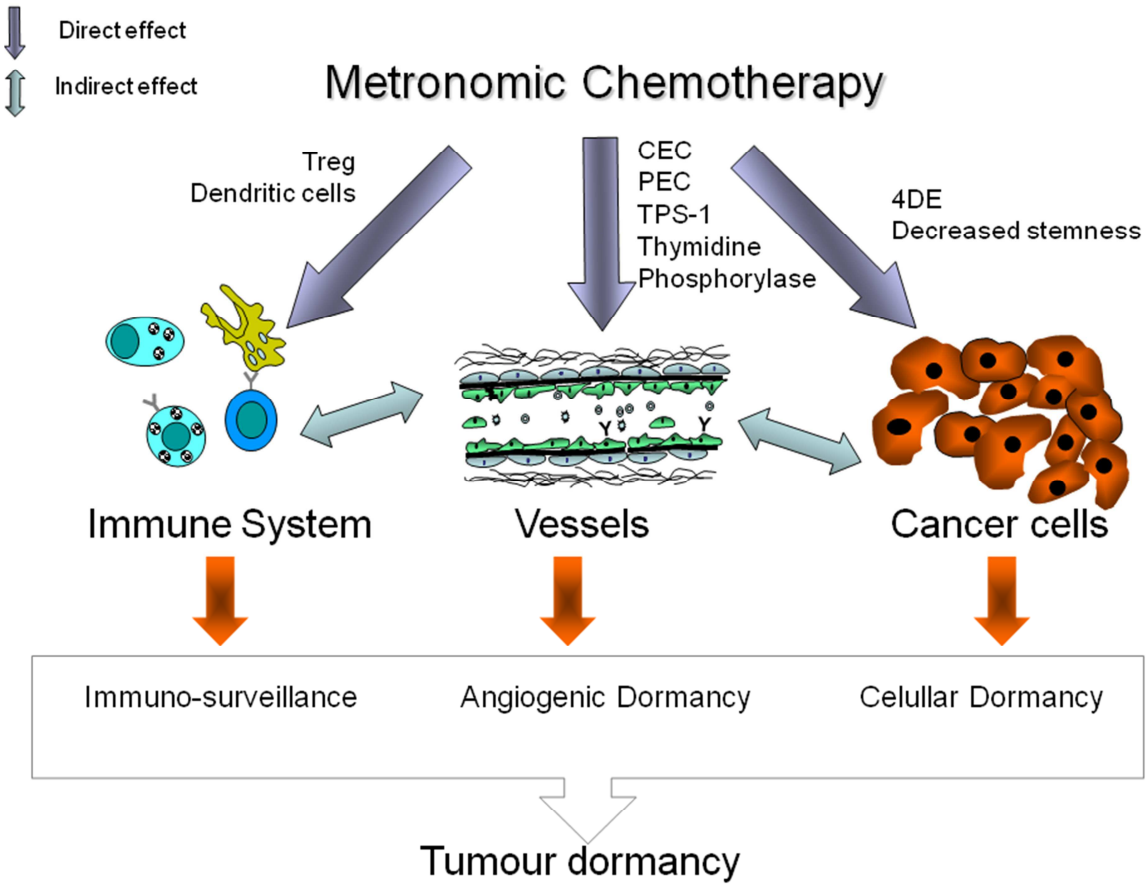
D. Mechanisms of Action

The description of various mechanisms of action of MC and its clinical applications may increase interest in metronomic scheduling. A summary of the potential anticancer effect of MC is shown in **D. Figure 1.**

D. Figure 1

Mechanism of actions of metronomic chemotherapy



Abbreviations used:

CEC, circulating endothelial cells; PEC, progenitor endothelial cells; TPS-1, thrombospondin-1; 4DE, drug-driven dependency and deprivation effect

D.1 *Classical Anti-Angiogenic Effect*

The classical paradigm explaining the mechanism of action of MC was initially proposed by Folkman and Kerbel[1,2]. By targeting tumor endothelial cells, MC might be able to indirectly destroy both sensitive and/or drug-resistant cancer cells by destroying exiting vessels and preventing neoangiogenesis leading to the initiation of hypoxia and starvation for nutrients. This theory has been confirmed by many pre-clinical and clinical studies. Nevertheless, it is not fully satisfactory. For instance, this paradigm cannot fully explain the occurrence of persistent stable disease (SD) even after the completion of metronomic treatment[3]. In addition, one of the initial rationales for the development of anti-vascular therapies and in particular, MC was based on the idea that their target, i.e., vascular endothelial cells, are genetically more stable, and thus, less likely to acquire drug resistance than cancer cells—a hypothesis that has since been demonstrated to be at least partially wrong as several studies have suggested that endothelial cells may develop resistance to long-term anti-angiogenic therapies[3,4] and that tumor endothelial cells can harbor cytogenetic and chromosomal abnormalities usually observed in cancer cells [3,5].

The processes contributing to angiogenesis in pediatric malignancies are to some extent comparable to those implicated in adult solid tumors[6]. These processes involve the following key elements:

1. VEGF (predominantly VEGF-A) and its receptors
2. Endogenous angiogenesis inhibitors such as thrombospondin-1 or angiostatin
3. Tumor stroma pericytes, signaling through platelet-derived growth factors (PDGF-A and PDGF-B) and their receptors
4. Endothelial cells and their circulating subsets:
    a. Circulating endothelial cells (CEC) that reflect the turnover of tumor vasculature

b. Circulating endothelial progenitor cells (EPC)

5. Integrins that are transmembrane receptors playing a role in endothelial cell adhesion and migration during angiogenesis

6. Cancer cells with receptors to ligands such as VEGF, PDGF, Stem Cell Factor (SCF), and others

So far, the anti-angiogenic effects of MC have been shown to be mediated depending on the drug or combination of drugs such as thrombospodin-1, thymidine phosporylase, CEC, and EPC.[3,7,8]

D.2 *Immunity*

Although the relevance of tumor immunology remains marginal in the treatment of most cancers, growing evidence indicates that anticancer immune responses may be crucial for the long-term control of cancer treated with chemotherapy[9]. Indeed, besides the well-known harmful side effects of chemotherapy on the immune system, such as neutropenia and lymphopenia, increasing number of preclinical and clinical studies suggest that some cytotoxic drugs such as taxanes, temozolomide, cyclophosphamide, and anthracyclines display important immunostimulatory effects[3,9]. In fact, some anticancer agents as well as radiotherapy can elicit immunogenic cancer cell death or immunostimulatory side effects, which can induce specific immune responses. The consequential anticancer immune responses can either help to eradicate cancer cells, which, for instance, have escaped chemotherapy or maintain a stable residual situation containing micrometastases in a stage of dormancy[3]. Recently, several reports have shown that MC was capable of inducing such an immune anticancer effect. Surprisingly, these effects have been discovered only almost 10 years after the description of MC but one must be aware that MC was originally described in nude mice, which have an altered immune system.

For instance, both metronomic TMZ and cyclophosphamide have been reported to stimulate anticancer responses through the depletion of CD4+CD25+ regulatory T cells (Treg)[10, 11] Bassini et al. studied the impact of various TMZ regimens on Treg cell population in a TMZ-resistant rat model of glioma. Interestingly, the metronomic regimen with TMZ was the only one capable of diminishing a percentage of Treg/CD4+ within tumors. Treg depletion induced by the low-dose metronomic TMZ regimen was accompanied by a decrease in suppressive function of the remaining Treg cells. Anyhow, Treg depletion alone was not sufficient to reduce tumor growth in a significant manner[10].

Elsewhere, Ghiringhelli [11] showed that cyclophosphamide could also decrease Treg-suppressed tumor immunity of established tumors from cell clones isolated from a rat colon carcinoma. Moreover, in patients with advanced cancer, metronomic cyclophosphamide could induce a specific reduction of circulating Treg cells[12] associated with the restoration of peripheral T cell proliferation and innate killing activities.

Therefore, metronomic regimen with cyclophosphamide or TMZ might not only affect tumor angiogenesis but also strongly diminish immunosuppressive Treg cells, which in turn improve the efficacy of anti-cancer chemotherapy regimens. It is very likely that part of this long-term and long-lasting efficacy of MC regimen containing TMZ or cyclophosphamide may rely on immune mechanisms.

Investigating several different kinds of anticancer agents (topoisomerase inhibitors, anti-metabolites, microtubule-targeting agents, and alkylating agents), Tanaka et al.[13] showed that some chemotherapeutic drugs, including paclitaxel, vinblastine, and etoposide, could induce dendritic cell maturation. Interestingly, if drugs like etoposide and vinblastine are used in metronomic way, they could promote dendritic cell maturation at non-toxic concentrations. Tanaka et al.[14] further validated the relevance of this immunostimulatory effect

in vivo by showing that tumoral injection of vinblastine at low doses triggers maturation of dendritic cells within the tumor and statistically increased anticancer effects.

D.3 *Tumor Dormancy*

Tumor dormancy is a phase occurring during the early phase of cancer before the triggering of the angiogenic switch[15] or after the completion of anticancer treatment during the remission phase where tumors can resume their growth. Tumor dormancy can take place from residual disease at the primary or at any metastatic site. Interestingly, tumors may also be dormant because of a treatment-driven re-induction of tumor dormancy. For instance, some cases in several metronomic studies displayed a persistent stable disease even after the completion of their treatment[16, 17, 18]. Many preclinical and clinical evidence indicates that cancer dormancy can be obtained by several non-exclusive mechanisms such as:

1. Cellular dormancy (G0-G1 arrest): The disturbance of cross talk between growth factors and adhesion signaling prevents tumor cells from interpreting information arising from their microenvironment, leading to cellular dormancy through a G0-G1 arrest. Cancer cells may be thus maintained in a quiescent state in a microenvironment-dependent manner resulting in cell growth arrest[3,19].

2. Angiogenic dormancy[3,19]: Tumor dormancy may be obtained through a new therapeutically forced equilibrium between pro- and anti-angiogenic cytokines resulting in tumor stabilization.

3. Immunosurveillance: Residual cancer cells can be kept clinically dormant by the immune system[20, 21]. For instance, Shirrmacher et al. used an animal tumor model to show that T cell immunity was essential for both the induction and maintenance of tumor dormancy [21]. Since an interruption of this dormancy state might be due to immune escape, the reinforcement of anticancer immunity by MC as discussed above could constitute an alternative mechanism promoting tumor dormancy.

D.4. *Getting Rid of Resistant Cells Through Growth Competition Between Tumor Population*

According to Gatenby[22] and his model named "adaptative therapy," the cost of developing resistance may slow the speed of growth of resistant cancer cells as compared to their parental/sensitive counterpart. In turn, when resistant and sensitive cancer cells grow together, there is a trend of repopulation with sensitive cells. These concepts may be illustrated with an increasing "re-treatment response" following a rechallenge with drugs that had already been used to treat a patient. For example, some non-small cell lung cancer (NSCLC) patients who respond well to treatment with epidermal growth factor receptor (EGFR) and tyrosine kinase inhibitors (TKIs) and who later experience therapy failure, demonstrate a second response to EGFR TKI re-treatment after a "drug holiday" [23,24]. Similar re-treatment responses are well established for several other anti-cancer agents[25] and have been reported with MC[26]. The possibilities of a rechallenge may rely on several mechanisms.

First, interactions between clones impact the overall sensitivity of the tumor. Indeed, Chmielecki et al.[27] recently confirmed the Gatenby hypothesis in a model of NSCLC that was tyronase kinase inhibitor sensitive or resistant. Indeed, the drug-sensitive and drug-resistant mutant cells exhibited different growth kinetics, with resistant cells growing slower.

When a combination of sensitive and resistant cells were grown together with a predetermined percentage of respective clones, populations with small percentages of resistant cells (1% and 10%) displayed similar sensitivity to erlotinib as parental cells (0%) did, whereas sensitivity was reduced when T790M clones made up >25% of the population.

Second, on long-term exposure to doses that mimic metronomic treatments with standard anticancer drugs like paclitaxel or etoposide, cell lines derived from prostate or colon cancer could lose their tumorigenicity or could even become non-tumorigenic when grafted in mice via epigenetic mechanisms as demonstrated by Yan et al.[24] Using a Darwinian finalistic perspective, these findings suggest that cancer cell populations use a dynamic survival strategy in which individual cells transiently assume a reversibly drug-tolerant state to protect the population from eradication by potentially lethal exposures to chemotherapy. These results unveil new potential of actions of MC: an anticancer effect on tumor initiating cells leading to a decrease in proliferative potential that may in turn contribute to cellular tumoral dormancy.

Third, the resistance that arises during MC seems to be different from resistance occurring during MTD. Indeed, resistance to metronomic scheduling of anticancer treatment (MSAT) can be transient as reported by Chmielecki et al.[27] and Yan et al.[24] which indeed paves the way for a re-challenge with the same agents. Moreover, Emmeneger et al. [31] recently reported that prostate cancer cells that were selected for resistant to metronomic cyclophosphamide did not induce cross resistance to other anti-cancer agents such as paclitaxel or doxorubicin.

Finally, we have previously proposed a new anticancer effect of MC based on the induction of chemotherapy-driven dependency of cancer cells followed by a forced drug deprivation-induced cell death called *drug-driven dependency-deprivation effect* or *4D effect* [3,29], which could allow killing of dependent (and the most resistant) cancer cells.

Overall, the recently described new mechanisms of action of MC urge to reconsider the MC model. This new model implies major changes in the initial paradigm according to which the anticancer activity of MC does not rely on the direct effect on cancer cells but only on an endothelial cell-based anti-angiogenic effect (**D. Figure 1**). Indeed, MC cannot only target endothelial cells but also cancer cells or cancer stem cells. This may occur through nutrition or oxygen starvation due to the destruction of cancer blood vessels. However, it may also occur through the immune system that can have its efficacy reinforced by MC. Additionally, depending on the agents used, MC can also act directly or indirectly on many cellular or extracellular stromal components such as activated fibroblasts, pericytes, macrophages, and mast cells depending on the agents used, which can hamper normal functions of the tumor microenvironment. This has led us to propose that multitarget metronomic scheduling therapy could be a logical and interesting development of MC.

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E. Clinical Development in Children

Published pediatric metronomic studies are reported in Table 1. We did not take into account studies that are retrospectively metronomic, such as those involving oral etoposide. These studies are mainly pilot, phase I or II studies. Overall, they are reports on over 200 children. Among these, 32 (14%) achieved complete or partial response and 89 (40%) a stable disease with a clinical benefit of over 50%. These studies confirm that the metronomic regimen can be safe and can control refractory disease. Large prospective phase II studies with several cohorts as well as randomized studies are ongoing and might help to further demonstrate the role of MC in children as maintenance and/or as salvage therapy.

E. Table 1
Clinical data about the use of metronomic chemotherapy in children.

Patients Population	nb patients	Metronomic Protocol	RR & CB%	Best reponses	Authors
brainstem glioma	15	temozolomide (oral daily for 6 weeks) + radiotherapy	RR : NA CB : 33%	NA	Sharp and al.
refractory or relapsing brain tumours	27	temozolomide (oral daily for 6 weeks)	RR : 15% CB: 33% RR	CR : 2 PR : 2 SD : 9 PD : 13	Baruchel and al.
refractory or relapsing neuroblastoma	21	zoledronic acid (IV 1/4 weeks) cyclophosphomide (oral daily)	RR : 15% CB: 33%	CR : 0 PR : 1 SD : 9 PD : 11	Russel and al.
refractory or relapsing tumours of various type	12	cylophosphomide (oral daily for 3 weeks) alternating with methotrexate (oral 2X/S for 3 weeks) celecoxib (oral daily) Vincristine (IV 1X/week every 8 weeks	RR: 0% CB: 58%	CR : 0 PR : 0 SD : 7 PD : 5	Fousseyni and al.
refractory or relapsing brain tumours	26	topotecan (oral daily 3/4weeks)	RR: 7% CB: 23%	CR : 0 PR : 2 SD : 4 PD : 20	Minturn and al.
refractory or relapsing tumours of various type	17	vinblastine (iv, 3 times/week) Celecoxib (daily oral)	RR: 0% CB:17%	CR : 0 PR : 0 SD : 9 PD : 8	Stempack and al.
	16	Cyclophosphamide (oral daily) Celecoxib (oral daily)	RR: 7% CB: 23%	CR : 0 PR : 0 SD : 7 PD : 16	
refractory or relapsing tumours of various type	20	Etoposide (oral daily for 3 weeks) alternating with cyclophosphamide (oral daily for 3 weeks) Thalidomide (oral daily) Celecoxib (oral daily)	RR: 15% CB: 50%	CR : 0 PR : 3 SD : 10 PD : 8	Kieran and al.
refractory or relapsing or "high risk of relapse" (age < 5 years)	10	Alternating combination every 3 weeks: celecoxib (oral daily) or retinoic acid (oral daily) and either oral daily temozolomide or cyclophosphamide	RR: 20% CB: 80%	CR : 0 PR : 3 SD : 10 PD : 8	Choi and al.
refractory or relapsing or "high risk of relapse" tumours of various type	17	Celecoxib (oral, daily) + etoposide (oral daily for 2 weeks) alternating with cyclophosphamide (daily oral for 2 weeks)	RR: 0% CB: 35%	CR : 0 PR : 0 SD : 12 PD : 5	André and al.
refractory or relapsing or "high risk of relapse" brain tumours tumours of various type	22	Etoposide (oral daily for 3 weeks) alternating with temozolomide (oral daily for 6 weeks) Celecoxib (oral daily) Retinoic acid (oral daily for 2 weeks)	RR: 32% CB: 77%	CR : 4 PR : 11 SD : 12 PD : 5	Sterba and al.
refractory or relapsing or "high risk of relapse" tumours of various type	16	cylophosphomide (oral daily for 3 weeks) alternating with methotrexate (oral 2X/S for 3 weeks) celecoxib (oral daily) Vinblastine (IV 1X/week for 7 weeks)	RR : 25% CB: 25%	CR : 3 PR : 1 SD : 0 PD : 12	André and al.
brainstem glioma	8	Toptecan (IV daily) for 6 weeks + Radiotherapy followed by thalidomide (oral daily) and celecoxib (oral daily) Etoposide (oral daily for 3 weeks)	RR : NA CB : 100%	NA	Kivivuori and al.

Abbreviations used:

RR, Response Rate; CB, Clinical Benefit; CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; NA, Not Available

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F. Future Perspectives

MC is currently undergoing several developments. Metronomic regimens are being tested in relapsing patients, but most importantly, MC is also being tested as a maintenance treatment in several tumors with high risk of relapse following the completion of first-line treatment. Last, new combinations of metronomic agents are being evaluated and integrate targeted compounds such as bevacizumab or motor inhibitors or are combined with the MTD chemotherapy.

F.1 *Combinations*

The first 2 metronomic publications by Browder and Klement[1,2] reported that the association of MC with anti-angiogenic molecules was more efficient than that with MC alone. It may also be very interesting to combine metronomic and MTD chemotherapy as proposed by Pietras et al. who showed that this combination leads to a survival benefit in the mouse model of cancer[3]. Most interestingly, very recently, Peyrl et al.[4] reported a metronomic combination with MC (etoposide and cyclophosphamide), drug repositioning (thalidomide and celecoxib), intrathecal therapy (pegylated-araC and etoposide), and anti-VEGF therapy in patients with relapsing medulloblastoma and obtained quite encouraging long-time event-free survival (EFS)[4]. Although a combination of MC with targeted agents will very likely be not affordable for low-income countries, the use of drug repositioning is a very interesting alternative.

F. 2. *Non Anti-cancer Chemotherapy Metronomic Agents*

Drug repositioning consists of the use of "old" drugs for new indications. The theoretical and pragmatic advantages of testing already established drugs for a potential effect on cancer cells are obvious: The side effects are known and have already been well documented. Phase I is not mandatory; therefore, these drugs can immediately enter phase II studies for testing their efficacy in cancer treatment. One of the main efforts of drug repositioning relies in identifying the right new therapeutic areas to prospectively test a given drug. Some examples are available in the field of cancer with drugs such as celecoxib[5-9], valproic acid [10,11], statins [12], metformin [13], itraconazole [14], or more recently, propranolol [15-17] and nifurtimox [18-20]. Interestingly, most of the time these agents display new mechanisms of action that can otherwise be achieved by using only expensive new developed agents.

These agents are given daily and can be regarded as metronomic [21]. Here, we will briefly focus on celecoxib, propranolol, and nifurtimox.

F.2.1 *Cox Inhibitors*

Celecoxib (Celebrex®) was initially developed as a selective cyclooxygenase-2 (Cox2) inhibitor for the treatment of chronic pain; Cox-inhibitors have now been widely used in oncology. Indeed, molecular studies have shown that the overexpression of Cox-2 is an important feature in malignant neoplasms and that Cox-2-derived prostaglandins participate in several cancer-associated processes such as carcinogenesis, inflammation, immune response suppression, apoptosis inhibition, angiogenesis, and tumor cell invasion and metastasis [5,6]. Thus, because of its low toxicity profile, daily administration of Cox-inhibitors is feasible (even twice a day); celecoxib has been frequently associated to other MC or MTD chemotherapy [6-8].

F.2.2 *Beta Blockers*

Beta-blocking agents could also be an interesting path to explore and possibly, to be used as metronomic therapy for cancer. Indeed, Léauté-Labrèze et al. reported the first small series of pediatric patients with refractory hemangiomas treated successfully with beta-blocking agents [15]. The potential anti-angiogenic effect of a well-known drug with a very favorable safety profile, especially as compared to common anticancer agents, is worth being considered. The underlying anticancer mechanism of action remains quite unclear; however, Sommers Smith et al. [16] showed that beta blockade stimulated apoptosis of endothelial cells when cultures of capillary endothelial cells were used. We recently showed that propanolol displayed both direct anticancer effects on several cell lines, including neuroblastoma cell line and an antiangiogenic effect in vitro. Interestingly, depending on the cell lines and

anticancer agents used, propranolol could have both its intrinsic, cytotoxic, and antiangiogneic effects strengthened by chemotherapy. These results were confirmed in vivo [17]. Recently, epidemiologic studies have also shown the potential clinical utility of beta blockers in patients with breast cancer [18]. However, further preclinical and clinical studies are warranted to further study the potential therapeutic effect of beta-blocking agents in children with cancer [24].

F.2.3 *Nifurtimox*

Elsewhere, following the results of a case report [19], the 5-nitrofuran, nifurtimox, a drug commonly used to treat Chagas disease, has recently been shown to exert potent anticancer effects against neuroblastoma both in vitro and in vivo, in part through the inhibition of TrkB signaling [20]. A recent phase I study not only defined the recommended dose of nifurtimox to be used in neuroblastoma patients but also provided important insights into its potential efficacy [21]. There is an ongoing phase II study to confirm the potential of nifurtimox in children with neuroblastoma or medulloblastoma (NCT00601003).

Overall, repositioned drugs, because of their well-know and well-established low toxicities, their low cost, and their new mechanism of action, allow innovative treatment techniques to be built.

F.3 *Maintenance Therapy*

One of the most obvious developments of MC is maintenance therapy. Maintenance therapy aims at maintaining the clinical benefit obtained so far by the treatment. One of the challenges of maintenance therapy is to preserve a good quality of life by displaying only low toxicity.

In adults, many examples illustrate the growing potential of maintenance therapy such as maintenance therapy with estrogen-receptor inhibitors in breast cancer. Indeed, Sanchez-Munoz et al.[22] or Orlando [23] agreed that a combination of metronomic cyclophosphamide and methotrexate was effective in heavily pre-treated breast cancer patients since the clinical benefit was observed in more than 15% of the patients with a median duration of response/disease stabilization of 21 months. Treatment was well tolerated. In prostate cancer, a maintenance treatment consisting of androgen blockade is a part of the standard treatment [24], and several studies have reported the interest of MC using cyclophosphamide alone [25] or in combination [26-27].

Thus, it seems that maintenance chemotherapy is a realistic strategy that can prolong time of progression in these patients. In pediatric tumor types, the benefit of maintenance therapy has also been observed. Recently, the long-term benefit of retinoic acid maintenance, in patients with high-risk neuroblastoma has been confirmed, regardless of previous intensification with high-dose chemotherapy and autologous hematologic stem cells rescue [28]. Sterba et al.[67] reported the efficacy of a multi-agent metronomic protocol in patients with neuroblastoma suggesting its potential interest, if given as maintenance treatment.

Recently, Klingebiel et al. reported, in children with metastatic soft tissue sarcoma, that conventional chemotherapy followed by oral maintenance therapy containing trofosfamide, etoposide, and idarubicin led to statistically significant better overall survival (OS) (58%) versus 24% OS in patients treated with conventional chemotherapy followed by high-dose chemotherapy with peripheral stem cells transplantation [32]. This has led to protocols for children with metastatic rhabdomyosarcoma incorporating a maintenance treatment for a year with vinorelbine and cyclophosphamide.

Overall, in tumors for which the role of maintenance therapy had already been established, it seems that MC offers new possibilities to further explore prolonged treatments.

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G. Metronomic Chemotherapy in Low-Resource Countries

Eighty percent of all children live in developing countries, and 200,000 of them are diagnosed with cancer each year. Unfortunately, these children have only very limited access to curative treatments and only about 25% survive. In high-income countries, approximately 75% of the 50,000 children diagnosed with cancer each year survive. Pediatric oncology has a rather low priority due to the relatively few number of cases, cost of treatments, and efficacy of treatments. Nevertheless, by promoting education, dedicated medical networks, organizing meetings, and setting minimum standards for training and care, pediatric cancer unit may improve not only cancer treatment but medical care in general [1,2].

Many problems prevent the development of an efficient management for children with cancer in low-resource countries (LRC), such as the availability of drugs and treatment facilities' cost of drugs; distance to the pediatric oncology unit; compliance with treatment; delayed diagnosis; lack of follow-up, especially in children who require surgery as the primary mode of treatment; earlier consultation with traditional practitioners; and cultural barriers [1-5].

So far, programs dedicated to children with cancer in LRC have mainly focused on curable diseases such as lymphoma [6], leukemia [7], Wilms tumor [8], or retinoblastoma [9]. Thus, one of the major burdens lies in the management of children with relapsed, progressive, and/or very advanced disease without any curative option. Treatment of these patients with second-line intensive or experimental treatments with new expensive drugs, as it is done in Europe or in the United States, is totally a non-realistic option in LRC [10, 11].

Therefore, MC appears as an attractive approach that can be used in patients in developing countries [10, 11]. Indeed, this strategy possesses at least 4 main advantages that are of the highest importance for patients living in these countries.

1. First, these treatments can display quite a low cost as they are mostly based on the use of old rather cheap generic drugs, for instance, 30 pills of cyclophosphamide cost around 10 Euros in European countries.
2. Second, these drugs are also usually available in oral form avoiding costly hospitalizations and intravenous (IV) injections and/or the use of central venous accesses that are both expensive and increase the risk of infection.
3. Third, oral treatments can be taken at home and therefore do not require long travels to care centers and costly hospitalizations; thus, may decrease abandonment of treatment.
4. Last, as MC demonstrates only limited toxicity, it will not expose children to higher risk of infections or nutrition problems.

Thus, overall, although long-term compliance with oral treatment remains an important issue, it seems that MC is worth being investigated in developing countries. Recently, the first pediatric experience was published by Fouseyiny et al.[12] who prospectively tested the role of a multidrug metronomic regimen and demonstrated tolerability and potential efficacy of such an approach. Indeed, among the 12 children treated with mainly relapsing Wilms tumors [6] and retinoblastoma [5], while no objective response was observed, 7 patients experienced disease stabilization and continued their treatment for 15 to 24 weeks. Most importantly, in 3 patients, the disease remained stable for at least 6 months after the completion of treatment. The MC regimen was well tolerated. A new study (Metro-Mali02) that used the Metro-Mali01 as a backbone, in which valproic acid as a histone deacetylase (HDAC) inhibitor has been added, is currently ongoing in Bamako. More details about this protocol can be found on metronomics.newethicalbusiness.org.

The potential interest of MC for children living in LRC has also recently been highlighted in India [13,14]. Indeed, Sondhi et al. reported a boy with medulloblastoma who was initially treated with gross total excision of the primary tumor followed by radiotherapy. Six years later, he developed disseminated bone relapse associated with bone marrow involvement. He was successfully salvaged with a combination of metronomic low-dose cyclophosphamide, etoposide, and zoledronic acid. During the third metronomic meeting that took place in Haifa in March 2012, Banavalli also reported the interest of an oral etoposide-cyclophosphamide-tamoxifen combination for relapsing sarcomas.

The Metronomics Global Health Initiative (www.metronomics.newethicalbusiness.org) was launched recently to raise awareness and to demonstrate the potential role of MC and drug repositioning for children with cancer living in developing countries and then promote its use. This needs to be demonstrated by state-of-the-art studies that respect ethical concepts [13]. "Best-buy" interventions with both short- and long-term health benefits should be prioritized. Although changing the course of severe diseases or the perception of certain types of treatment may not seem cost effective at first sight, it may not only impact people's immediate health but also affect mentalities and ultimately lead to the biggest and most sustainable changes. The key point here is to avoid the unrealistic "copy and paste" approach adopted by the richer countries and to develop novel, constraint-tailored therapeutic strategies. A rising number of cancer patients in developing countries, for instance, can pave the way for changing mentalities and exploring new context-adapted metronomic treatments. It will, however take new global health models involving all stakeholders (NGO, pharmaceutical industries, governments, and communities) to allow low-income countries to develop their own way to treat their people suffering from cancer.

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H. Conclusion

More than 10 years have passed since the first description of MC. Although, low-dose chemotherapy had already been used for decades with, for instance, oral etoposide or cyclophosphamide, in palliative settings or methotrexate and 6-mercaptopurine in the maintenance phase of acute lymphoblastic leukemia (ALL), only recently, it has been experienced that MC could indeed be efficient in patients with either refractory diseases or given as a maintenance therapy in patients with high-risk solid tumors. Besides, the newly described mechanisms of action of MC allow us to design future MC regimens more rationally by scheming metronomic protocols with several agents using rotation and/or breaks and using agents that should elicit an anticancer immune response. The advantages associated with MC make it attractive alternative strategies for children living in LRC. Preliminary evidence is available. State-of-the-art phase II and III studies associating MC and drug repositioning are mandatory to demonstrate its efficacy. It will, however take new global health models involving all stakeholders (NGO, pharmaceutical industries, governments, and communities) to allow low-income countries to develop their own way of treating their people suffering from cancer.