maximum tolerated dose) might optimize its antiangiogenic properties. In fact, the results of preclinical and clinical studies have been encouraging, especially when drugs are used in combination with new molecularly targeted agents such as specific angiogenesis inhibitors. By targeting normal, terminally differentiated and genetically stable ECs, the risk of acquired drug resistance might be reduced. Through the use of metronomic chemotherapy, it may be possible to re-evaluate not only the re-treatment of refractory cancers with agents that have previously failed, but also agents that have traditionally been considered inactive or ineffective against a particular type of cancer. Moreover, as some of the most useful agents tested in both *in vitro* and *in vivo* models include vinca alkaloids, taxanes, and antimetabolites, the principle of this concept does not necessarily require the development of so-called "magic bullets".

At present, however, it remains difficult to determine the optimal doses and schedules for metronomic chemotherapy, as a rapid objective response is absent, and identification of MTD using conventional toxicity criteria is relatively straightforward. Also, it is still unclear whether metronomic chemotherapy actually exerts an antiangiogenic effect that contributes to putative antitumor efficacy, and there is currently no standard for monitoring and quantifying the angiogenic response during therapy. Nevertheless, from a clinical view point, metronomic chemotherapy appears promising, not only because it reduces toxicity and the need for growth-factor support or anti-emetic drugs, but also because it may improve antitumor effects. The actual significance of this concept should be confirmed by much larger, prospective, and randomized controlled trials that compare metronomic dosing chemotherapy schedules with conventional ones. In addition, novel functional or angiogenesis-specific markers and new high-resolution techniques for blood flow imaging and analysis are essential for monitoring of angiogenesis.

INTRODUCTION

The concept of standard chemotherapy is to administer an agent at a dose close to the maximum that a patient can tolerate before the onset of unacceptable toxicity. This is based on the precept that tumor cells represent the prime target of chemotherapy and that eradication can be achieved only through repetitive administration of a conventionally derived maximum tolerated dose (MTD). On the other hand, recent evidence suggests that most chemotherapeutic agents are capable of inhibiting tumor angiogenesis by targeting tumor endothelial cells (ECs), and that metronomic, frequent or continuous administration of the same agent at low doses might optimize its antiangiogenic properties [1-4]. These findings have broken out of the paradigm shift of anticancer chemotherapy, and now tumor ECs have become an important target of chemotherapy. In this article, we summarize the current status of knowledge about the antiangiogenic properties of metronomic, continuous low-dose chemotherapy.

CURRENT CONCEPTS OF THE TUMOR GROWTH MODEL AND STANDARD CHEMOTHERAPY

MTD-based chemotherapy is based largely on the "much is better" concept (Table 1). This was originally derived from kinetic modeling of curative chemotherapy response of murine leukemia L1210 cells *in vitro*, which are a cell line showing rapid and almost exponential growth fraction closed to 100% as a consequence of frequent cell cycling [5,6]. In this model, the cytotoxic effects of chemotherapy follow a log-kill kinetics model whereby a given dose always kills a certain fraction, rather than a certain number, of exponentially growing tumor cells. This model is attractive because of its simplicity, and thus modern chemotherapy regimens are usually designed to kill as many tumor cells as possible by treating patients with the highest doses possible without causing life-threatening levels of toxicity, *i.e.* the MTD. In general, host toxicity is often only marginally less than anti-tumor efficacy, creating a narrow therapeutic index, and the agents are usually administered in a pulsed manner with breaks (usually of 3 to 4 weeks) between cycles to allow recovery of normal dividing cells of rapidly regenerating tissues such as bone marrow and gut mucosa, at the sacrifice of hair-follicle cells. These regimes are not only associated with unpleasant or serious adverse effects that compromise the quality of life for the patient who is undergoing therapy, but also provide a chance for tumor regrowth during the resting period. The apex of this concept is high-dose chemotherapy with the use of autologous hematopoietic stem cell support.

MTD-based chemotherapy has led to a profound increase in the survival of children and adults with certain types of cancer, whereas responses for the majority of solid tumors, particularly when they are advanced or associated with metastatic disease, are short lived, and neither complete tumor eradication nor sustained regression is often achieved. In this respect, it is important to note that the MTD concept was developed using synchronized, log-phase non-mutagenic cells and non-cell-cycle-specific agents for which chemotherapy was optimized to affect all cells in culture. Therefore, this concept is unlikely to reflect the nature of most solid tumors, which consist of heterogeneous cell populations, where only cells at a specific stage of the cell cycle are likely to be affected by chemotherapy. In addition, genetic and epigenetic variations within a heterogeneous population of tumor cells might result in diversity of drug sensitivity, thus eventually conferring refractoriness to conventional chemotherapy. For these reasons, most human solid tumors do not exhibit purely exponential growth, but rather appears to show non-exponential Gompertzian kinetics [7]. In this model, the ratio of proliferating cells to total cells decreases with increasing tumor size, the growth fraction declines exponentially over time, and a plateau phase can be observed in some malignancies. As a result, the tumor growth curve has three distinct regions; a slow initial phase, a middle phase characterized by rapid growth, and a slow plateau phase. Another important consideration in this model is the impact of residual tumor cells on tumor growth after chemotherapy. In volume-reduced Gompertzian cancer models, regrowth of tumor cells between cycles of chemotherapy is more rapid than in exponential models, and is fastest at small tumor volumes.

Table 1. Comparison of several concepts of chemotherapy

	Conventional chemotherapy	Dose-dense chemotherapy	High-time chemotherapy	Metronomic chemotherapy
Primary aim	Total tumor cell kill	Total tumor cell kill	Maximum tumor cell kill	Dormancy status
Dosage	MTD-based	MTD-based	MTD or DLT of longer exposure, lower-dose schedule	1/3 to 1/10 of MTD
Dose intensity	High	High	Low	Low
Interval	Intermittent (usually 3 weeks)	Intermittent (usually 1-2 weeks)	Daily / continuous	Continuous (daily / week)
Duration	Short term	Short term	Long term	Long term
Projected target	Tumor cells	Tumor cells	Not defined (Endothelial cells for low dose)	Endothelial cells
Endpoint in clinical study	Established	Established	Feasibility of long term administration	Not established
Levels of toxicity	High	High	Low	Low
Quality of life	Impair	Impair	Maintain	Maintain
Resistance	Frequent	Frequent	Not defined	Rare
Need for growth factor support	Sometimes	Frequently / mandatory	Available	No
Combination with anti-angiogenic or targeted drugs	Available	Available	Available	Available

Abbreviations: DLT, dose-limiting toxicity; MTD, maximum tolerated dose.

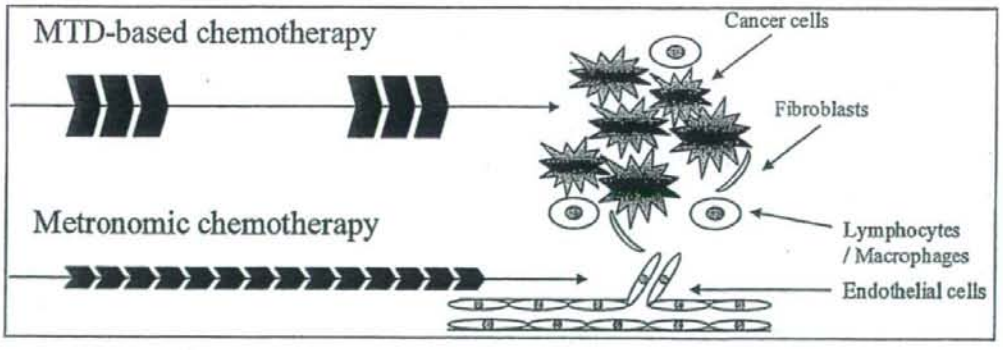

Fig. 1. Target of standard chemotherapy and metronomic chemotherapy. Tumor tissue consists of tumor cells and stroma including endothelial cells. The standard chemotherapy mainly targets tumor cells, while metronomic chemotherapy targets tumor endothelial cells. The size of the bullets and interspaces refer to the doses and interval of chemotherapy, respectively.

Based on this concept, Norton [8] has proposed that administration of an appropriate and effective dose of chemotherapy more frequently would limit tumor regrowth between cycles and minimize the possibility of acquired chemoresistance with a higher probability of logarithmic tumor-cell destruction. This approach is referred to as dose-dense chemotherapy,

and is usually designed so that at least the same amount or, more commonly, even a greater total amount of drug can be administered over time to reach the highest possible dose, resulting in an increase of dose intensity (DI). In agreement with this hypothesis, it has been reported that weekly dosing of paclitaxel involving delivery of lower individual, but more frequent doses of paclitaxel was effective for the treatment of advanced breast cancer [9]. Another example is the Intergroup Trial C9741 / Cancer and Leukemia Group B (CALGB) Trial 9741. This trial was designed to test two concepts - dose density and sequential therapy - based on experimental data and mathematical reasoning, and demonstrated that dose-dense chemotherapy improved survival relative to standard chemotherapy in an adjuvant setting for patients with node-positive breast cancer [10].

In either case, however, the MTD concept targets tumor cells themselves, and does not take into account the importance of individual tumor components including tumor cells and stromal tissue containing ECs [11-13], despite the fact that angiogenesis is a hallmark characteistics of cancer, and that tumor growth and metastasis are dependent on continued angiogenesis (Fig. 1).

ALTERNATIVE CONCEPT OF CHEMOTHERAPY

In chemotherapy, from a basic pharmacological viewpoint, the principles of response lag (threshold), response slope and plateau are evident, and the influence of exposure duration is unmistakable [14]. Indeed, the often-cited formula for cell killing, e^{-kct}, has both a concentration (c) and a time (t) component in the exponent, and thus one can hypothesize that once a killing effect has been achieved with a maximal dose, then no further efficacy can been expected, the only remaining variable is time. Recently, the importance of scheduling has been rediscovered as a new "old" concept of chemotherapy, termed high-time chemotherapy [2]. This chemotherapy approach seeks the maximum drug exposure time for a given drug concentration. For example, when an effective dose of a drug can be maintained for 14 days with little and reversible toxicity, we can either increase the daily dose during this period, or extend the administration period of the same dose to 21 or more days until the longest possible time that results in dose-limiting toxicity (DLT). Although this concept is useful for long-term therapy, careful assessment of the pharmacokinetics and pharmacodynamics of the drug involved would be required [4].

On the other hand, since recent evidence has suggested that dividing ECs are present in the growing blood vessels formed in tumors [15], there is now a shift in thinking towards the view that either continuous infusion or frequent, even daily, administration of chemotherapeutic agents at doses significantly below the MTD with no prolonged drug-free breaks would be more effective, not only in terms of reducing certain toxicities but perhaps even improving the antitumor effect as well, by shifting the target of the chemotherapy from the tumor cells to the vascular ECs [2-4]. This concept was first referred to by the term "metronomic chemotherapy" by Hanahan *et al.* [3], while the term "antiangiogenic dosing" or "high-time for low dose chemotherapy" have been advocated by others [2,16,17]. In either case, targeting normal, terminally differentiated and genetically stable ECs may have a theoretical advantage in avoiding, or at least delaying the onset of acquired resistance which,

in part, occurs because of the massive and diverse genetic instabilities present in tumor cells. In other words, drug resistance could be reversed simply by shifting the focus of the treatment from the tumor cells to still-sensitive tumor ECs. This is supported by the fact that the maximum level of myelosuppression observed in cancer patients remains unchanged after multiple cycles of chemotherapy, even when the tumors in these patients have become drug-resistant [18]. If normal bone marrow progenitor cells acquire resistance to chemotherapy in the same way that genetically unstable, highly mutable tumor cells do, then myelosuppression would gradually decline and disappear.

In addition to direct effect on ECs, several studies have indicated that an endogenous inhibitor of angiogenesis, thrombospondin 1 (TSP1), is a potent mediator of the effect of metronomically administered cyclophosphamide (CPA) [19-21]. TSP1 blocks the proliferation of, and induces apoptosis in ECs by binding to CD36 receptors, which are expressed by ECs [22]. TSP1 can also bind and sequester vascular endothelial growth factor (VEGF), and thus block its proangiogenic activity. Moreover, a proapoptotic siganal elicited by TSP1 generates CD95L, a ligand for the CD95 death receptor [23]. In addition to CPA, several other chemotherapeutic agents such as cisplatin, docetaxel, and doxorubicin could induce endothelial CD95 in vivo and in vitro at low doses that do not kill ECs [24,25]. In contrast, as metronomically administered CPA retains its efficacy in mice that are unable to produce the other endogenous angiogenesis inhibitors endostatin and tumstatin [19], these molecules might not be involved in the antiangiogenic effect of this type of chemotherapy. Thus, metronomic chemotherapy might not necessarily act directly on ECs, but might exert its effect by inducing the EC-specific inhibitor TSP1 [26,27].

Interestingly, the metronomic approach does not appear to be confined to chemotherapy. For example, tumor response to radiotherapy is regulated not only by tumor cell phenotype, but also by EC apoptosis. In a study by Garcia-Barros *et al.* [28], antitumor effects of irradiation were mediated through a primary event involving damage or destruction of the tumor neovasculature, followed by the death of tumor cells surrounding the affected vessels, indicating that microvascular sensitivity regulates tumor cell response to radiation within the clinically relevant dose range. In fact, radiation is sometimes administered at a lower than normal dose, known as hyperfractionated radiation. Moreover, in a study by Slaton *et al.* [29], daily administration of smaller doses of interferon (IFN)-alpha was significantly more effective than larger doses once a week in a mouse cancer xenograft model. IFN-alpha is also effective for treatment of pediatric patients with hemangiomas or giant-cell tumors when administered in small daily doses over prolonged periods of time [30,31]. Similarly, metronomic photodynamic therapy has been shown to be useful in a malignant brain tumor model [32,33].

On the other hand, more compressed or accelerated schedules of metronomic chemotherapy might be viewed as a variation of dose-dense chemotherapy. However, metronomic chemotherapy treats cancer as a chronic disease in which the tumor burden is maintained at the lowest achievable level over a long period, whereas the latter aims for complete tumor cell killing based on the MTD-concept, as mentioned above. Thus, metronomic chemotherapy is a different approach from dose-dense chemotherapy for the management of cancer, and the concept of metronomic chemotherapy is supported by several mathematical modeling studies [34,35].

Reasons for Ineffective Antiangiogenic Activity of MTD-Based Chemotherapy

As tumor ECs are not quiescent, and moderately high fractions of proliferating ECs can be detected during tumor angiogenesis [15], dividing ECs should be vulnerable to the cytotoxicity of chemotherapeutic agents. However, there might be some reasons why MTD-based chemotherapy does not effectively target ECs. First, MTD-based chemotherapy usually necessitates breaks in drug administration to allow bone marrow recovery, and thus damage to ECs could be largely repaired during such rest periods. In fact, in a study by Browder *et al.* [16], CPA was shown to cause apoptosis of ECs when administered at the MTD in immune-competent syngeneic mice. In this study, the first cells in the tumor to undergo apoptosis were the ECs, although this antiangiogenic effect was of no significant therapeutic benefit.

In association with this, it is important to note that chemotherapy could mobilize circulating endothelial progenitors cells (CEPs) from the bone marrow into the circulation, and these mobilized CEPs would be recruited into the tumor vasculature [34,36,37]. In fact, a recent study using two human lymphoma xenograft models has demonstrated that low-dose metronomic dosing of CPA caused a sustained reduction of viable CEPs, whereas MTD-based administration caused a marked drop followed by a rapid rebound during the break period [38]. Thus, the prolonged interval between chemotherapy cycles would provide an opportunity for new ECs to be recruited, either from existing vasculature or from CEPs, allowing angiogenesis to proceed [38-40]. Alternatively, the ECs might be protected from damage or cell death induced by chemotherapeutic agents by high local concentration of EC survival factors such as VEGF, basic fibroblast growth factor, and thymidine phosphorylase [41-43].

Thus, little therapeutic benefit might be derived from this transient antiangiogenic effect, and in fact, conversly, endothelial recovery during the treatment-free period might support the regrowth of tumor cells [16]. This concern has been shown to be justified in a pivotal trial conducted in patients with metastatic colorectal cancer; survival was improved in patients receiving irinotecan, fluorouracil, and lecovorin with the anti-VEGF agent bevacizumab, in comparison with patients who did not receive bevacizumab [44]. Adding bevacizumab might alter the tumor growth pattern, improve intratumoral chemotherapy delivery, and result in improved rates of minimal residual disease and possibly disease eradication. This finding is consistent with the Norton-Simon hypothesis that addition of any effective anticancer agent to chemotherapy could improve results by limiting tumor growth between chemotherapy cycles, as tumor regrowth requires the development and maintenance of tumor vasculature and the recruitment of a blood supply [45].

Preclinical Evidence of Metronomic Chemotherapy

The strategy of metronomic scheduling of chemotherapy to exploit its capacity to inhibit angiogenesis was first supported by experimental evidence that the use of a lower dose of CPA (approximately one-third the MTD for mice) with more frequent weekly administrations

reduced the risk of acquired drug resistance in mice bearing Lewis lung carcinoma and L1210 leukemia cells [16]. This strategy facilitated tumor eradication by denying any opportunity to repair the damage inflicted on the vasculature. It is also interesting that mouse tumors which had previously acquired resistance to CPA administered using a conventional MTD schedule responded dramatically over time to the same drug when a lower dose, with a more frequent schedule, was started, and addition of the angiogenesis inhibitor TNP-470 to the antiangiogenic schedule of CPA eradicated drug-resistant tumors. The same study also confirmed that metronomic dosing of a number of other cytotoxic agents including 5-fluorouracil had an antiangiogenic effect.

Similarly, Klement *et al.* [46] demonstrated that the mitosis-blocking cytotoxic agent vinblastine directly killed cultured ECs at doses considerably below those required to affect drug-sensitive neuroblastoma cells, and that intraperitoneal administration of low-dose vinblastine well below the MTD with a continuous schedule impaired tumor growth in mice. The same study also explored the use of the monoclonal antibody DC101 to disrupt the function of the VEGF type 2 receptor (VEGFR), which is highly expressed on activated ECs. Interestingly, the combination of the antiangiogenic vinblastine with DC101 resulted in complete and sustained regression of established neuroblastoma xenografts in mice, while DC101 alone resulted in significant but transient tumor regression. Histopathological assessment revealed that vinblastine alone and DC101 alone both increased the width of the apoptotic rim, suggesting that cells most distal to the tumor vasculature were primarily affected, although a large percentage of tumor cells still remained viable in the tumor center, and despite an evident increase in apoptosis, net tumor growth was observed. In contrast, in the combined therapy group, there was overwhelming loss of both cell viability and preexisting tumor architecture. Vascular damage was common to all the treatment groups, but the prevalence and severity of the damage were greatest in the combination treatment group. In another study, the same group also demonstrated that continuous low-dose (one-tenth of MTD) chemotherapy was highly effective against orthotopically grown multidrug-resistant human breast cancers in mice with severe combined immunodeficiency disease, but usually only when the drugs were used in combination with DC101 [47]. In this study, paclitaxel and vinblastine exhibited better therapeutic profiles than other drugs including cisplatin or doxorubicin, mainly because of their lack of cumulative toxicity such as weight loss.

Moreover, Man *et al.* [48] found that low-dose CPA administration in drinking water was a safe and convenient treatment with significant antitumor efficacy in xenotransplanted Swiss nude mice carrying tumors derived from human breast, colon and melanoma cell lines. This study clearly demonstrated that per os drug in human could be chronically and safely administered in a similar but more convenient basis in mice through drinking water, and similar to the previous studies, the effects of continuous administration of CPA was enhanced by DC101.

Collectively, these studies have established that a metronomic chemotherapy dosing schedule can be antiangiogenic, achieve a durable and potent tumor response, and also provide a stable and safe way to circumvent multidrug resistance, simply by shifting the focus of the treatment away from the drug-resistant tumor cell population to the drug-sensitive tumor ECs. At the same time, these studies have demonstrated the value of using angiogenesis inhibitors in combination with the main therapy. These results have since been

confirmed by others with or without combination with targeted drugs such as thalidomide, PEX fragment of metalloprotease-2, platelet factor fragment PF-4, anti-endogrin antibody, TSP1 peptide, endostatin, anti-VEGFR, kinase inhibitors (imatinib, SU5416, SU11248), metalloprotease inhibitor (BB-94, BAY 12-9566), immunotherapy, or hyperthermia [24-26,49-64].

Among them, Hermans *et al.* [58] assessed a combination of metronomic administration of CPA with specific antitumor immunotherapy eliciting cytotoxic T lymphocytes (CTLs) reactive to recombinant epitopes expressed by the murine melanoma model B16.F10, and found that this combination strategy dramatically enhanced antitumor responses over either therapy alone. Both metronomic chemotherapy and MTD-based chemotherapy deleted the proliferating tumor-specific CTLs in blood, although the deletion showed slower kinetics with the metronomic schedule. This study demonstrated the potential benefits of combining non-immunosuppressive metronomic dosing of CPA with an immunotherapeutic approach to treat cancer. Moreover, in a study by Ma *et al.* [63], metronomic dosing of gemcitabine and cisplatin significantly attenuated the disruption of the homeostatic balance between tissue factor and the tissue factor pathway, suggesting that metronomic chemotherapy might reduce adverse clotting events associated with chemotherapy alone or in conjunction with antiangiogenic drug combination therapies. In association with this, it is interesting to note that, in a study by Emmenegger *et al.* [65], long-term daily low-dose CPA administration caused neither significant toxicity to bone marrow and gut mucosa normally affected by MTD-based chemotherapy of the same agent, nor had a negative impact on wound healing.

Moreover, a study of particular interest investigating the "chemo-switch" protocol, a MTD-based chemotherapy followed by metronomic chemotherapy as maintenance therapy, demonstrated that the inhibition of platelet-derived growth factor receptor signaling by the tyrosine kinase inhibitors imatinib and SU11248 enhanced the efficacy of metronomic chemotherapy in a transgenic mouse model of pancreatic neuroendocrine cancer [59]. Treatment with imatinib reduced the pericyte coverage of tumor blood vessels, rendering ECs more sensitive to chemotherapy. Moreover, using a combination of imatinib and SU11248 with metronomic chemotherapy elicited regression of established tumors and prolonged the survival of the mice. By combining an initial MTD-based chemotherapy with maintenance therapy consisting of metronomic chemotherapy and SU11248, remarkable efficacy was achieved in terms of objective response and survival. Importantly, this study highlights the significance of pericytes as a target of antiangiogenic therapy, and validates the chemo-switch regimen. In addition, in a study by Shaked *et al.* [64], repeated administration of bolus doses of CPA every 3 or 6 weeks, combined with a daily oral low-dose metronomic regimen, improved efficacy and significantly delayed the progression of transplanted PC-3 human prostate cancer xenografts, syngenic transplanted EMT-6 breast tumors, and "spontaneous" murine erythroleukemia. In this study, the bolus dose and low-dose metronomic chemotherapy caused a sustained reduction in the viability of CEPs. Thus, standard MTD-based "remission-induction" chemotherapy and long-term metronomic chemotherapy might not be mutually exclusive, and could be used in a beneficial and harmonious manner [27].

CLINICAL PRECEDENTS OF METRONOMIC CHEMOTHERAPY

A retrospective view of medical science reveals that there are actually many clinical precedents for these preclinical findings. For example, some of the most successful treatment regimens in pediatric oncology resemble metronomic chemotherapy in that they involve daily administration of low doses of cytotoxic agents over prolonged periods of time as maintenance therapy. In fact, in the protocol for acute lymphoblastic leukemia, children are treated with low doses of oral methotrexate on a weekly basis and 6-mercaptopurine on a daily basis for up to 3 years [27,66]. In this regard, it is interesting to note that drugs used in this setting have been demonstrated to be antiangiogenic [67-69]. In addition, daily administration of CPA and weekly administration of vinca alkaloids such as vincristine or vinblastine has been shown to be effective for treatment of infants with neuroblastoma [70], and weekly chronic chemotherapy using vincristine and actinomycin, or vincristine, actinomycin and CPA, for nearly half a year has also been used successfully for the treatment of pediatric patients with rhabdomyosarcoma [71]. Moreover, the pediatric regimen (CPA, doxorubicin, vincristine and prednisone), which is used for treatment children with non-Hodgkin's lymphoma is far less intensive in the use of vincristine and prednisone than the regimen for adult, and is conceptually similar to metronomic chemotherapy [27].

Similarly, a variety of chemotherapeutics resembling an antiangiogenic dosing schedule has also been documented for adult solid and hematological tumors. For example, in a study comparing the classical CPA, methotrexate, and 5-fluorouracil (CMF) schedule with a modified 3-week intravenous CMF schedule for postmenopausal patients with advanced breast cancer, the classical regimen resulted in a significantly better response rate and survival, possibly because, in the classical CMF schedule, CPA is administered orally on days 1-14, followed by 2 weeks rest, which more closely resembles an antiangiogenic schedule than intravenous administration of CPA every 3 weeks. Similarly, oral administration of uracil and tegafur (UFT) and leucovorin daily for 28 days every 35 days in patients with metastatic colorectal cancer was reportedly well tolerated, without causing neutropenia and oral mucositis that complicates intravenous schedules of 5-fluorouracil plus leucovorin [72]. In this context, it is important to note that oral administration of UFT is commonly used to treat a wide variety of solid tumors in Japan [73,74], and the usefulness of oral UFT has also been demonstrated in an adjuvant setting in patients with colon cancer or breast cancer [74-76].

Moreover, intuitive physicians have found that significant proportions of patients with breast and ovarian cancer who had stopped responding to MTDs of a taxane given once every 3 weeks, subsequently responded to the same drug once it was switched to a weekly schedule at about one third of the MTD [77-79]. These weekly schedules using lower doses were found to minimize the toxicities associated with the once-every-3-week MTD regimen [78-82]. Similar results have been observed in patients refractory to a bolus MTD dose of agents such as etoposide and CPA when the same agent was administered chronically at a lower dose [83]. Furthermore, there are several other situations in which prolonged oral administration of relatively low doses of chemotherapeutics is already in use for palliative-like regimens that are less toxic and are sometimes used to treat elderly patients who are less able to cope with MTD-based chemotherapy. These agents include etoposide [84,85], an

antiangiogenic topoisomerase II inhibitor, razoxane [86], an alkylating agent, temozolamide [87-89], a prodrug of a bifunctional alkylating agent, treosulfan [90], and an alkylating oxazaphosphorine, trofosfamide [91]. Among them, razoxane was initially developed as a potent antiangiogenic agent, and was later found to be a noncleavable inhibitor of topoisomerase II. Similarly, low-dose methotrexate plus vinblastine has been administered every 7-10 days for several months in a substantial subset of patients with advanced, inoperable aggressive fibromatosis [92]. Although it is has not yet been demonstrated conclusively whether these regimens described above actually have antiangiogenic activity that contributes to their antitumor efficacy, the primary importance of these studies appears to be that a metronomic-like schedule of chemotherapy is feasible and of potential clinical benefit.

Clinical Studies of Metronomic Chemotherapy

Several clinical studies including case reports, retrospective studies, feasibility studies and phase II studies have evaluated the possible benefits of metronomic chemotherapy regimens (Table 2). In most of these, CPA has been involved, and administered orally on a daily basis, sometimes for up to 2 years, with no break periods, and in some cases, oral low-dose methotrexate has also been given on two consecutive days on a weekly basis. For example, the phase II study conducted by Colleoni *et al.* [93] evaluated low-dose oral CPA (50 mg/day) administered on a daily basis and oral methotrexate (2.5 mg, bd) administered twice a week in 64 patients with progressive, advanced and refractory breast cancer, and achieved an overall response of 32% with no high-grade toxicity. In this study, serum VEGF levels decreased two months after the start of treatment, although there was no significant difference in the median reduction between responders and non-responders.

On the other hand, Globe *et al.* [94] performed a chart review to evaluate the efficacy and toxicity of continuous oral administration of a combination of CPA (50 mg/day) and dexamethasone (1 mg/day) in patients with prostate-specific antigen (PSA) progression despite hormone therapy and antiandrogen withdrawal. Dexamethasone, in addition to its other properties, has been shown to exert an antiangiogenic effect, possibly by through inhibiting the production of VEGF [95,96]. They reported that almost 70% of the patients showed a decrease in serum PSA levels of 50% or more, and that the duration of response was 8 months with well tolerated toxicities.

Similarly to preclinical studies of metronomic chemotherapy, several studies have combined metronomic chemotherapy with targeted drugs such as cyclooxygenase-2 inhibitors (celecoxib, rofecoxib), the peroxisome proliferator-activated receptor gamma agonist (pioglitazone), thalidomide [97-105], and more recently, designated angiogenesis inhibitors such as bevacizumab [27,106]. It is also interesting to note that metronomic chemotherapy was found to be cost effective in terms of outcome and resource utilization when low-dose CPA and methotrexate chemotherapy was compared with published phase II studies using oral or intravenous chemotherapy in a palliative setting for previously treated metastatic breast cancer [105].

Table 2. Reported studies of metronomic chemotherapy.

Reporter, year	Patient population, setting	Treatment	Outcome
Colleoni M, 2002 [93]	Metastatic breast cancer, phase II study (n=64)	Low-dose CPA (50 mg/day) PO, and low-dose MTX (2.5 mg bd, twice a weekly) PO	2 CR , 10 PR 10, 8 SD for at least 24 weeks, 9 grade 3 liver toxicity, 12 grade 2 leukopenia, decrease of serum VEGF level at 2 months
Glode LM, 2003 [94]	Advanced hormone refractory prostate cancer, retrospective study (n=34)	Low-dose CPA (50 mg/day) and low-dose dexamethasone (1 mg/day)	22 CR, 2 SD assessed by prostate specific antigen response, duration of treatment 9 months
Spieth K, 2003 [97]	Advanced melanoma, pilot study (n=12)	Low-dose oral treosulfan (500 mg/day) , and cyclooxygenase-2 inhibitor, rofecoxib (25 mg/day)	1 PR, 4 SD (12-36 weeks), median survival time 13 months, no grade 3, 4 toxicities
Vogt T, 2003 [98]	Advanced and pretreated but progressive, malignant vascular tumors, pilot study (n=6)	Oral pioglitazone (45 mg/day), rofecoxib (25 mg/ day), and after 14 days, oral trofosfamide (3 x 50 mg/day)	CR 2, 1 PR, SD 3, median progression-free survival 7.7 months, mild toxicities
Coras BC, 2004 [99]	Endemic Kaposi sarcoma, a case report (42 years old)	Same as the regime reported by Vogt	PR for 18 months, no significant toxic effects
Nicolini A, 2004 [100]	Metastatic hormone refractory prostate cancer, case series (n=8)	Low-dose oral CPA (100-150 mg/day, alternately)	2 PR, 3 SD, 3 PD, grade 2, 3 neutropenia in all patients
Reichle A, 2004 [101]	Previously treated metastatic melanoma or soft tissue sarcoma, phase II study (n=40)	Same as the regime reported by Vogt	4 CR, 2 PR, 5 SD for more than 6 months, no grade 3, 4 toxicities
Fassas AB, 2005 [102]	Refractory Waldenstrom's macroglobulinemia, a case report	Low-dose chemotherapy with continuous intravenous CPA and adriamycin, and rituximab, dexamethasone, bortezomib, thalidomide, rapamycin, interferon-gamma	Minimal lymphomatous involvement in bone marrow, stable monoclonal markers, normal platelet count, marked functional improvement
Herrlinger U, 2005 [103]	Recurrent glioblastoma, phase II (n=10)	Oral low-dose CPA (100 mg/day) and MTX (5mg twice weekly)	No CR, no PR, prematurely closed because progression-free survival rate at 6 months was 0%
Kieran MW, 2005 [104]	Recurrent or progressive pediatric cancer, feasibility study (n=20)	Thalidomide, celecoxib with alternating oral etoposide and CPA every 21 days for 6 months	3 PR, 40% of patients completed 6 months of treatment, correlation of baseline level of thrombobospondin-1 and prolonged response, no response pattern in VEGF, bFGF, endostatin
Bocci G, 2005 [105]	Metastatic breast cancer, comparative pharmacoeconomic evaluation	Low-dose CPA and MTX	Metronomic chemotherapy was significantly cost-effective

Abbreviations: bFGF, basic fibroblast growth factor; CPA, cyclophosphamide; CR, complete response; DLT, dose-limiting toxicity; PO, per os; SD, stable disease; PD, progressive disease; PR, partial response; MTX, methotrexate; VEGF, vascular endothelial growth factor.

CURRENT ISSUES AND FUTURE DIRECTIONS OF METRONOMIC CHEMOTHERAPY

Since Polverini *et al.* [107] first reported the antiangiogenic effect of conventional chemotherapy as early as 15 years ago, virtually every class of chemotherapeutic agent has been shown to have antiangiogenic activity, as determined by *in vitro* and *in vivo* assays, using multiple potential criteria for antiangiogenicity, including differential cytotoxicity to ECs at doses that have no effect on tumor cells themselves, interference with EC function without EC death, interference with a specific portion of the angiogenic cascade, and *in vivo* evidence of antiangiogenic activity [1]. However, some agents have profound antiangiogenic effects at very low doses, others exhibit antiangiogenic activity only at near-maximum cytotoxic concentrations, whereas others have striking antiangiogenic activity in one xenograft model but none in others. Moreover, only a few agents have been demonstrated to produce substantial antiangiogenic activity *in vivo*, while several cytotoxic agents have been shown to affect ECs *in vitro* [4]. In contrast, metronomic dosing of some agents could stimulate VEGF-mediated angiogenesis. In a study by Albertsson *et al.* [108], metronomic cisplatin and fluorouracil treatment significantly stimulated angiogenesis in a dose-dependent, non-linear manner *in vivo*. The proangiogenic effect occurred frequently in the case of cisplatin and infrequently in the case of fluorouracil. Thus, there might be a finely tuned dose-effect situation, and no set criteria could actually define the antiangiogenic activity of cytotoxic agents.

From the viewpoint of clinical practice, there are a number of challenges to devising metronomic chemotherapy regimens in terms of the choice of agents, dosing schedule, and study design. For example, as rapid objective responses are absent and identification of MTD using conventional toxicity criteria is relatively straightforward for metronomic chemotherapy, selection of an optimum antiangiogenic dose that is nontoxic but still effective remains difficult. In addition, the critical pathways by which EC biology can be manipulated have not been identified *in vivo*, and levels of circulating angiogenesis regulators have not proven particularly effective as markers of antiangiogenic therapy [109-111]. Thus, there is still no standard for monitoring and quantifying the angiogenic response during therapy. Therefore, the approach of metronomic chemotherapy remains largely empirical. In fact, the dose for metronomic chemotherapy has been empirically determined to be 10 – 33% of conventional doses, in a similar way to conventional chemotherapy where the starting dose of a phase I study is empirically determined to be 1 to 0.1 of the LD_{50} obtained in a preclinical, animal-based study [112, 113]. In this respect, it is interesting to note that in a recent study by Shaked *et al.* [114], levels of CEPs or circulating ECs (CECs) correlated well with the degree of tumor angiogenesis and the response to VEGFR-targeted antiangiogenic therapy. In another study, the same group also found that each optimum biologic dose was strikingly correlated with the maximum reduction in viable CEPs [115]. Thus, monitoring of CEPs or CECs might provide a sensitive index [114,116-119], whereby the highest dose that can be administered given in a metronomic manner would be the dose exerting the greatest effect on ECs without clinical bone marrow perturbation [112,115]. Another promising possibility would be to determine the optimal dose level for a drug by detecting the circulating levels of TSP1.

In summary, metronomic chemotherapy appears to be promising, not only because it reduces toxicity and the need for growth factor support or anti-emetic drugs, but also show better anti-tumor effects. When MTD-based chemotherapy is indicated, however, it should not be changed just for the sake of increasing the antiangiogenic efficacy of any given agent until the actual significance of the metronomic concept has been confirmed by much larger, prospective, and randomized controlled trials comparing metronomic dosing schedules with conventional chemotherapy. It is also important to clarify which agents work best for metronomic chemotherapy, which combination or sequence with other modalities such as antiangiogenic agents, molecular-targeted agents or immunotherapy are more effective for the treatment of different tumor entities, or what mechanisms of resistance might develop over time. In theory, endocrine therapy might be an additional choice for advantageous combination with metronomic chemotherapy, as recent studies have indicated that 17 beta-estradiol modulates VEGF and VEGFR expression [120,121]. Moreover, novel functional or angiogenesis-specific markers and new high-resolution techniques for blood flow imaging and analysis are vital for monitoring antiangiogenic activity and response *in vivo*. Finally, careful monitoring of long-term or delayed adverse effects of metronomic chemotherapy will be necessary.

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