Guido Bocci
Giulio Francia
Editors

Metronomic Chemotherapy

Pharmacology and Clinical Applications

Springer

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Preface

Metronomic chemotherapy has arrived. In 2014 we are beginning to learn of phase III clinical trial results on this form of cancer treatment, defined by the continuous low-dose administration of conventional chemotherapy drugs. The emerging clinical data will be of paramount importance in allowing us to assess the impact of metronomic chemotherapy on different tumor types. Indeed, it is likely that the next decade will see a major reassessment of the possible applications of metronomic chemotherapy for the management of cancer. Furthermore, the clinical trial results will likely profoundly affect the direction of the preclinical laboratory efforts that are going on in parallel, aimed at improving this therapeutic strategy.

This book is our attempt to compile the history of metronomic chemotherapy that has brought us to this pivotal moment. In our view, it is important to learn from the successes, as detailed in the various chapters of this book, which allowed the transition of metronomic chemotherapy from the lab to the clinic in under two decades. It is also imperative that we catalog any errors and missteps that occurred along the way, so that their repeat may hopefully be avoided in the future, either by researchers studying metronomic chemotherapy or conceivably those developing entirely different therapeutic strategies. Insofar as this book is a catalog of what went right and what went wrong in developing a new therapeutic concept, we hope that it will be of interest to a general readership.

Scientists and clinicians caring for advanced cancer patients are well aware of the challenges in dealing with these diseases. Although metastatic cancers are responsive to a range of conventional cytotoxic agents, they generally recur and prove to be fatal. In facing the major obstacles to improvements in cures, the expectation today is that a different approach to preclinical and clinical research on chemotherapy will translate into genuine progress in routine therapy. While there is still much to be understood about the molecular mechanisms and pharmacology of metronomic chemotherapy, we are already seeing progress in the clinical use of this innovative therapy. Indeed, at the end of the 1990s, researchers such as Robert S. Kerbel and Judah Folkman began to investigate the antiangiogenic activity of frequent administration of low-doses of chemotherapeutic drugs, as a mechanism potentially contributing to their antineoplastic activity in vivo. During the past decade, a number of studies have shown that such metronomic chemotherapy has preclinical and clinical activity that may be ascribed to various mechanisms of action, including the inhibition of angiogenesis and the induction of an increase in

the immunological response. Oncologists, such as Marco Colleoni in Milan, have established numerous phase I and phase II clinical trials, investigating the use of metronomic chemotherapy in different tumors, and phase III clinical trials are currently ongoing. It is likely that the rapid acceptance of metronomic chemotherapy as a novel and interesting therapeutic strategy owes much to the almost parallel development of the concept in the lab and in the clinic.

The chapters of this book analyze all aspects of this new therapeutic approach and its possible future development. After an opening section on the pharmacodynamic bases of metronomic chemotherapy, including its antiangiogenic effects and impact on immunity, preclinical studies on some classes of drug are discussed. Clinical applications of metronomic chemotherapy in a wide variety of tumors are then addressed in detail, including description of the results of published studies. Where there are diverse views on what constitutes metronomic scheduling in the clinic, we tried to ensure that this book incorporates all points of view. The clinical pharmacology of metronomic chemotherapy is also considered in depth, encompassing pharmacokinetics, pharmacogenetics, and adverse drug reactions. Since cost of care is essential both in drug development and in the availability of treatment, there is also a chapter on pharmacoeconomics. The book ends with a description of the role of this therapy in the veterinary clinic, which we believe is an area of increasing importance in the development of most new therapies for cancer. The book's target audiences are oncologists, pharmacologists, veterinarians and translational researchers in the field of cancer.

This remains an incredibly exciting time for metronomic chemotherapy. The leaps in knowledge in the molecular and pharmacological aspects of this novel approach, and the clinical results, have been objectively remarkable. We hope you find the information contained in this book useful, guiding your translational research and everyday practice. Clearly new information continues to emerge on a monthly basis, and this of course is encouraging. And yet, we believe that there is a role for a book that provides a concise overall picture of metronomic chemotherapy, especially in 2014, which is a particularly eventful year for treatment developments that should result from the ongoing phase III clinical trials.

Metronomic chemotherapy was borne out of the ideas of Judah Folkman and Robert Kerbel, the pioneering laboratory work of Timothy Browder, and the early clinical studies by Marco Colleoni and colleagues. Two of those four pioneers have contributed to this book. Tragically, Dr. Folkman and Dr. Browder are no longer with us, and they missed out witnessing the results of the phenomenal translational work they initiated. This book is dedicated to their memory. Several chapters in the book are from researchers that were instrumental in the early years at the turn of this century in defining the concept of metronomic chemotherapy, and in highlighting its versatility as a treatment strategy. We apologize for any researchers that we may have omitted for reasons of time or space in the preparation of this book.

Finally, over the last year a team consisting of editors and authors have diligently worked to have this book published in its present form. We would like to use this

opportunity to thank each and every one of these contributors for the wonderful job you have done. Without their help and support we would have not been able to publish this book.

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Part I

Pharmacological Bases of Metronomic Chemotherapy

1 Development and Evolution of the Concept of Metronomic Chemotherapy: A Personal Perspective

Robert S. Kerbel

Abstract

The concept of metronomic dosing and scheduling of conventional chemotherapy drugs was first published in 2000, based on preclinical findings. Tentative validation for the treatment concept has now been obtained based on randomized phase III clinical trial testing. Most promising applications of metronomic chemotherapy may be in the maintenance treatment setting after induction therapy, using oral chemotherapeutic drugs, especially when combined with certain types of targeted agents such as VEGF pathway inhibiting antiangiogenic agents. A personal account of the historical development of the metronomic chemotherapy concept is summarized along with suggestions for improving its impact as a promising means of achieving better and less toxic cancer control, not only in patients in low and middle income countries, but also patients in highly developed high income countries as well.

Metronomic chemotherapy, a term first coined in 2000 by Douglas Hanahan and colleagues in an editorial commenting on two preclinical papers [1], one from Dr. Judah Folkman's lab [2] and the other from my own [3], generally refers to the close, regular administration of chemotherapeutic drugs – often daily – at individual doses that are well below the maximum tolerated dose (MTD) and administered or taken orally over prolonged periods of time. This investigational way of chemotherapy is fundamentally different from most conventional chemotherapy protocols, which often involve bolus administration of MTDs separated by long intervening breaks – usually in the order of 3 weeks. Such breaks are required when giving such higher doses of drug to allow recovery from the acute toxic side effects of chemotherapy administered in this conventional fashion, particularly myelosuppression. The aforementioned two preclinical studies quickly resulted in the initiation of a

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number of phase II clinical trials and later phase III metronomic chemotherapy-based clinical trials, which in turn recently led to the announcement of what may be the first prospective randomized phase III clinical trial result validating the clinical benefits of metronomic chemotherapy [4]. Nevertheless, the concept of metronomic chemotherapy has remained somewhat of a niche therapeutic concept, for which there are a number of reasons, as discussed in this review. First, I provide a summary of how the initial preclinical studies evolved; this summary is written from a very personal, and perhaps some might suggest, biased perspective.

I first became interested in antiangiogenic therapy around 1990 on the basis of an idea I had that was later published in 1991 [5], namely, that antiangiogenic drugs might not be vulnerable to acquired drug resistance over time because of the nature of the cellular target of such therapy. Thus, the final target is newly forming blood vessels in tumors and hence, in the main, the host vascular endothelial cells comprising such vessels, rather than the tumor cell population per se being nourished by such vessels. The assumption was that such host endothelial cells would be "normal," i.e., genetically stable, and would therefore lack the genetic derangements ("chaos") that characterize tumor cells – which are well known to be major drivers of acquired drug resistance to virtually any type of anticancer therapy [5]. Unfortunately, this hypothesis has not been validated in that acquired resistance to antiangiogenic drugs is now a common clinical phenomenon, and indeed, there have been a very large number of diverse mechanisms which have been proposed to account for the development of such antiangiogenic drug resistance [5–7]. The first one reported actually came from my lab [8], as did the first review on the topic [9].

In the aforementioned theoretical *BioEssays* paper [5], I also discussed the possibility that conventional cytotoxic chemotherapy drugs should have direct vascular targeting and hence antiangiogenic effects. This idea was based on the known presence of dividing endothelial cells in newly forming angiogenic blood vessels and the assumption that such cells – similar to other types of normal dividing cells in the body – should be susceptible to the cytotoxic and antiproliferative effects of chemotherapy. In other words, chemotherapy might represent an example of what I later termed "accidental antiangiogenic drugs" [10], and as such this might be an additional mechanism by which chemotherapy could ultimately (i.e., secondarily) cause antitumor effects – in addition to direct tumor cell killing or inhibition of cell proliferation [5]. I personally discussed these ideas with Dr. Folkman in late 1990. He was actively involved at the time in trying to develop antiangiogenic drugs such as TNP-470, and I put forward the notion that perhaps researchers and clinicians like him had for decades been doing antiangiogenic therapy without actually realizing it, simply by administering chemotherapy. However, I also pointed out what appeared to be a fact that was inconsistent with this hypothesis, and that is that there are types of cancer known to be highly angiogenic and yet intrinsically refractory to virtually every known common chemotherapeutic drug [5]. Renal cell carcinoma is probably the best example of this.

An insightful and clever resolution to this paradox was subsequently provided by Dr. Folkman's group almost a decade later. In a landmark study [2], Timothy Browder working in Dr. Folkman's lab reported results in which the potential of chemotherapy to function as a de facto antiangiogenic treatment could be optimized and sustained. Browder et al. found that a drug such as cyclophosphamide administered at MTDs

and schedules, involving 2-week breaks, could cause apoptosis of endothelial cells present in the blood vessels populating transplanted mouse tumors – but not in the blood vessels present in normal organs elsewhere [2]. However, the potential of this antiangiogenic "side effect" was apparently reversed during the 2-week drug-free break periods necessary after administering a cycle of the MTD cyclophosphamide protocol. Apparently, some kind of unknown host-mediated repair process was taking place to replace the damaged or dying endothelial cells caused by the chemotherapy so that the antiangiogenic effect was very transient and rapidly lost. Therefore, Browder et al. reasoned that shortening the break periods would compromise this hypothetical host repair process and enhance as well as sustain the antiangiogenic effects of the chemotherapy [2]. Naturally, this necessitated using lower doses than the MTD each time cyclophosphamide was administered. A more condensed (weekly) schedule was designed utilizing this strategy, and indeed, it was found to cause far greater levels of endothelial cell apoptosis in the tumor vasculature, and this in turn was associated with profound antitumor effects compared to the MTD protocol [2]. In addition, Browder et al. reported that tumors that acquired resistance over time in vivo to the conventional MTD chemotherapy could regain responsiveness to the same drug, e.g., cyclophosphamide, by switching from the conventional dosing and scheduling protocol to the new investigational protocol involving more frequent (condensed) administration of drug using lower individual doses [2].

As a result of these findings, Browder et al. coined the term "antiangiogenic scheduling of chemotherapy" to describe their findings, which were published in April 2000 [2]. I first became aware of this work as a result of Dr. Folkman openly discussing and sharing his findings with me roughly 2 years before they were published and approximately 18 months before he began disclosing the results publicly at scientific meetings. Upon first hearing the results, I became excited about the possibility that this way of giving chemotherapy might be especially ideal for combination with targeted antiangiogenic drugs that were in development at the time. Many such drugs were designed to interfere with the VEGF pathway of angiogenesis. Moreover, by 1998 it was just becoming apparent that such drugs, and others, were likely going to fall victim to the problem of acquired drug resistance. Therefore, combining a vascular targeting/antiangiogenic treatment strategy involving low-dose continuous chemotherapy, i.e., what was called "antiangiogenic chemotherapy" or "metronomic chemotherapy," might improve the effects of antiangiogenic agents such as those that target the VEGF pathway, and likewise such antiangiogenic drugs might improve the benefits of the investigational metronomic chemotherapy regimen. Moreover, such a combination would be less toxic, thereby allowing patients to receive it for long periods of time – a possibility ideal for maintenance or adjuvant therapies and for elderly or very young cancer patients.

We thus began studies immediately, with Dr. Folkman's knowledge, evaluating the combination of a drug called DC101, an anti-mouse VEGFR-2 antibody with the investigational metronomic chemotherapy regimen involving doses of vinblastine well below the MTD for mice, administered every 3 days, to treat very large established transplanted primary human neuroblastomas in SCID mice [3]. The protocol was devised by Dr. Giannoula Klement, a pediatric oncologist working in my lab as a postdoctoral fellow, in collaboration with Dr. Sylvain Baruchel, a pediatric

oncologist and researcher at Sick Children's Hospital in Toronto. This combination proved remarkably effective compared to either DC101 treatment alone or the "low-dose" investigational vinblastine chemotherapy protocol given as a monotherapy. Of note, this combination treatment was actually preceded by treating the tumor-bearing mice "upfront" for 3 weeks with higher cumulative doses of vinblastine administered through an infusional pump in order to debulk the large primary tumor, which was then followed by a regimen involving chronic administration of the condensed vinblastine low-dose therapy plus DC101 every 3 days [3]. Essentially this constituted a version of upfront higher-dose chemotherapy followed by a form of "maintenance" but less toxic chemotherapy. This is important with respect to some recent clinical results evaluating metronomic chemotherapy, as discussed below.

An aspect of this work worth mentioning is the pivotal role of ImClone Systems, Inc., and two researchers at ImClone at the time, Dr. Daniel Hicklin and Dr. Peter Bohlen, played in making the studies possible. Generous supplies of DC101 were made available to us for years, and significant annual financial support in the form of a sponsored research agreement was also arranged, which was proposed by ImClone, not by me, to help facilitate the proposed studies. This generosity was critical to the success of our initial and many subsequent studies of metronomic chemotherapy. Both Dr. Klement and Dr. Baruchel have since continued their studies on metronomic chemotherapy [11–17].

Considering that we initiated our studies in 1998, several years after Dr. Browder in Dr. Folkman's lab had initiated his visionary studies, it came as a surprise that our paper was published at the same time in April 2000 as Dr. Folkman's. How could this be? The reason is that Dr. Folkman told me that his paper had been rejected by at least three different high-impact journals before it was finally accepted by *Cancer Research*. In contrast, our paper was accepted rather quickly by the *Journal of Clinical Investigation* around the same time as Dr. Folkman's, with only minor revisions required. Back then papers accepted for publication by *J Clin Invest* were published much more rapidly than by *Cancer Research* which meant our paper would likely "scoop" Dr. Folkman's. As a result, I decided to try and hold back publication of our results in the *J Clin Invest*, by taking much more time than necessary to make the requested minor revisions, so that the two studies would be published simultaneously. I recall at the time having a conversation with the then science editor of the *J Clin Invest* – Dr. John Ashkenas – asking him to consider delaying publication of our paper in *J Clin Invest* so that it would appear in the literature at the same time as Dr. Folkman's *Cancer Research* paper. Needless to say, he was taken aback by this request! I recall that he said that he used to receive numerous calls from investigators asking if he would arrange to speed up publication of an accepted manuscript – but never to slow down publication! It was Dr. Ashkenas who suggested that I take more time than necessary to make the minor revisions requested so that the two papers would eventually come out together, and this is what I did. In the end, I think it was good that the two papers were published simultaneously as this made a stronger case for the counterintuitive notion that "less is more," i.e., findings that appeared to go against the entrenched dogma that the best or only effective way to go with chemotherapy is using MTDs separated by long break periods. Dr. Folkman always felt simultaneous publication was good and was incredibly gracious about this even though his group has started their work several years before us.

As mentioned above, the editors of the *J Clin Invest* requested Dr. Hanahan to write an editorial commenting on the contents of the two papers with the aim of trying to encapsulate the notion of giving lower-dose chemotherapy more frequently with no long break periods. Dr. Hanahan thought of the term "metronomic," i.e., the notion of close regular "beats" of chemotherapy like the regular beats of a metronome. There are deficiencies with this terminology, it could be said, and Dr. Folkman preferred his term of "antiangiogenic chemotherapy." However, I respectfully disagreed with him as I always felt that it would be unlikely, in the fullness of time, that giving chemotherapy in the lower-dose more frequent fashion would cause antitumor effects only through an antiangiogenic type of mechanism. And indeed, it was already well known at the time that administering very low doses of cyclophosphamide to mice could stimulate the immune system [18–22], something I found out after publication of our paper in the *J Clin Invest*. In addition, there was the additional obvious possibility that lower doses of chemotherapy given chronically could still cause in some way direct tumor cell inhibitory or even cytotoxic effects. The term "metronomic" did not imply any one particular or dominant mechanism and therefore I preferred it over "antiangiogenic chemotherapy." I believe that in this particular instance, I was correct since it is now increasingly accepted that metronomic chemotherapy involves several mechanisms, in addition to or even instead of antiangiogenesis [23].

1.1 That Was Then. What Happened Next, and What About Now?

After publication of the two papers from Dr. Folkman's lab and my own in 2000, there were a number of follow-up studies by a number of investigators, primarily evaluating the antiangiogenic basis of metronomic chemotherapy [23, 24]. The initial studies, as mentioned above, showed evidence of endothelial cell apoptosis/death in the tumor neovasculature. However, it seems that chemotherapy can have a dual and opposite effect on angiogenesis, i.e., either stimulate it or inhibit it – or both, in a temporal manner – another reason for avoiding the term "antiangiogenic chemotherapy." For example, in collaboration with Francesco Bertolini we reported, first in 2003 [25] and then in 2006 [26] and 2008 [27], that when chemotherapy is administered at MTDs which, as already mentioned, can cause direct endothelial cell apoptosis in the tumor neovasculature, i.e., a potential antiangiogenic effect, this way of giving chemotherapy can also cause a host effect involving the rapid and marked mobilization of bone marrow-derived cell populations (BMDCs) including endothelial progenitor cells (EPCs) which can then home to the chemotherapy-treated tumors, where they take up residence and promote tumor regrowth/repopulation during the break period following chemotherapy. For example, the presumptive EPCs can incorporate into the damaged angiogenic tumor vasculature and thus presumably replace some of the dead or dying endothelial cells caused by the chemotherapy treatment. As a result, this could be viewed as a proangiogenic (or "pro-vasculogenic") effect of the chemotherapy. Importantly, in this regard, we found that using a VEGF-pathway-targeting drug such as DC101 could block this BMDC host response and thus prevent this secondary proangiogenic effect mediated by the MTD

chemotherapy [26], thus maximizing or preserving the initial antiangiogenic effect of the same MTD treatment [26]. We also reasoned that it was this response that constituted the hypothetical host repair process responsible for reversing the antiangiogenic effects of MTD chemotherapy that Dr. Folkman's group suggested was taking place during the long successive drug-free break periods. In addition, this process remains, in my view, one way to explain how an antiangiogenic drug such as bevacizumab can enhance the effects of conventional chemotherapy – by blunting the proangiogenic/vasculogenic host BMDC/EPC response [26, 28]. In contrast, when giving chemotherapy in a metronomic fashion, Francesco Bertolini, with whom we collaborated, first reported in 2003 that the acute mobilization of EPCs is not only prevented, but in fact is actually targeted [25]. We collaborated with Dr. Bertolini on this work using cyclophosphamide [25] in a protocol involving giving the drug continuously, daily through the drinking water [29], a protocol developed by Shan Man, a senior technician in my lab, and then later extended the findings using other chemotherapeutic drugs [30]. Together these studies suggested that administering a metronomic chemotherapy regimen with an antiangiogenic biologic targeting, the VEGF pathway would cause maximal inhibition of this bone marrow host response in addition to causing greater degree of endothelial cell apoptosis in the tumor vasculature, as we and Browder et al. reported in 2000 [2, 3].

The basis for the property of metronomic chemotherapy to target activated endothelial cell and EPCs, at least in part, was worked out by Dr. Guido Bocci, a postdoctoral fellow from Italy in my lab at the time who showed metronomic chemotherapy could induce expression of thrombospondin-1 (TSP-1) in vitro and in vivo, a known inhibitor of angiogenesis [31], findings that were later confirmed by others such as Hamano et al. [32]. Yet another way that metronomic chemotherapy can conceivably cause an antiangiogenic effect, at least with certain drugs such as topotecan, a topoisomerase 1 inhibitor/poison, or the anthracycline adriamycin is by suppression of the expression of HIF-1α – as originally reported by Melillo and colleagues [33–40] and later by Greg Semenza's group [41]. HIF-1α is a known driver of angiogenesis – including VEGF-mediated angiogenesis [42], but also a factor responsible for resistance to antiangiogenic drugs [39, 40].

These and other findings – many by other groups [23] – bolstered the hypothesis that metronomic chemotherapy caused antitumor effects by inhibiting tumor angiogenesis. But a number of other findings seemed to cast doubt on this being the sole or in some cases even the major mechanism for it causing antitumor effects. As already stated, there was the existing literature that low doses o*f* certain chemotherapeutic drugs – especially cyclophosphamide – could cause stimulation of cytotoxic T cells by targeting T regulatory cells [43, 44]. This finding will likely assume ever-increasing importance in the near future, given the recent dramatic clinical successes of immunotherapy treatment strategies [45], e.g., single agent immune checkpoint control antibodies such as anti-PD1 or anti-CTLA4 antibodies [46]. In other words, combining metronomic chemotherapy with such immunotherapeutic drugs may be an ideal combination treatment strategy, and there is growing preclinical evidence to support this combination treatment strategy [47, 48], though it may require some adjustments in how to sequence the metronomic chemotherapy with

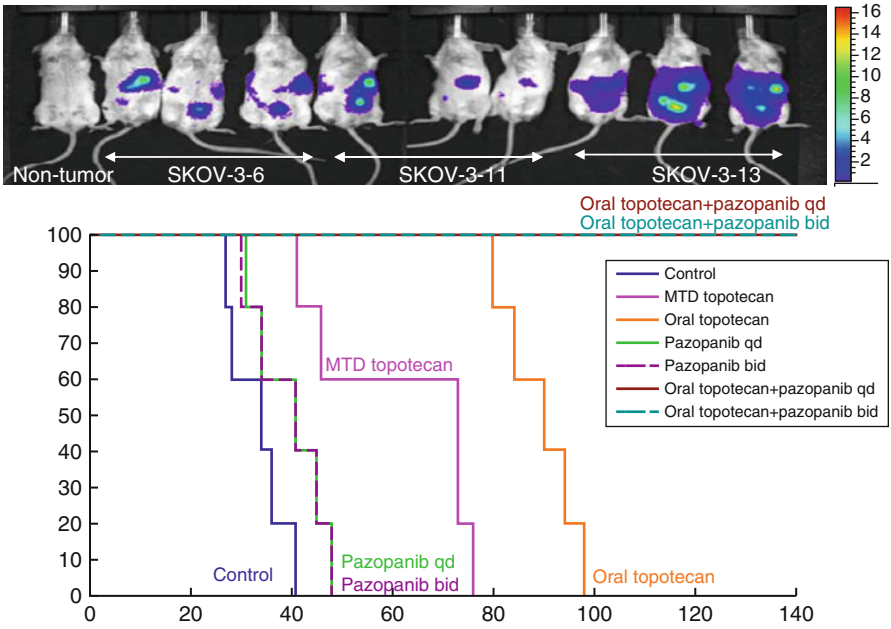

Fig. 1.1 Impact of chronic daily metronomic oral topotecan chemotherapy plus pazopanib in a model of advanced ovarian cancer. SCID mice were injected with 3×10^6 SKOV-3-13 luciferase-tagged cells given intraperitoneally 14 days after therapy was initiated as indicated. MTD topotecan refers to maximum tolerated dosing of the drug, i.e., 1.5 mg/kg for 5 days every 3 weeks given intraperitoneally. Oral topotecan refers to a metronomic regimen given at 1 mg/kg by gavage every day with no breaks. Once a day (q.d.) pazopanib refers to 150 mg/kg of the drug given by gavage, whereas twice a day pazopanib (b.i.d.) refers to the drug being given at 25 mg/kg each time by gavage. SKOV-3-6, SKOV-3-11, and SKOV-3-13 refer to different selected clones of luciferase-tagged SKOV-3 cells. The most aggressive clone, SKOV-3-13, was selected for further therapy studies as it gave rise to more aggressive multifocal disease in the peritoneal cavity (Taken from Hashimoto et al. [57])

the immunotherapy protocol as the work of David Waxman and colleagues have shown or suggested [49].

Regarding the notion that metronomic chemotherapy may also have significant direct antitumor cell effects, evidence for this possibility stems from several different considerations. First, resistance can eventually develop to metronomic chemotherapy, and there are some preclinical studies showing that this resistance is expressed by the treated tumor cell population [50]. Much of the work of resistance to metronomic chemotherapy has been done by Dr. Urban Emmenegger, a former postdoctoral fellow in my lab [51–56], and also Dr. William Cruz-Munoz [50], another former postdoctoral fellow. By way of example, treatment of human ovarian cancer in mice with metronomic oral topotecan plus an antiangiogenic agent, namely, pazopanib, can lead to extremely potent long-term antitumor effects [57], as shown in Figs. 1.1 and 1.2, and if the therapy is terminated, relapsing tumors begin to emerge, and such tumors are no longer responsive to the combination

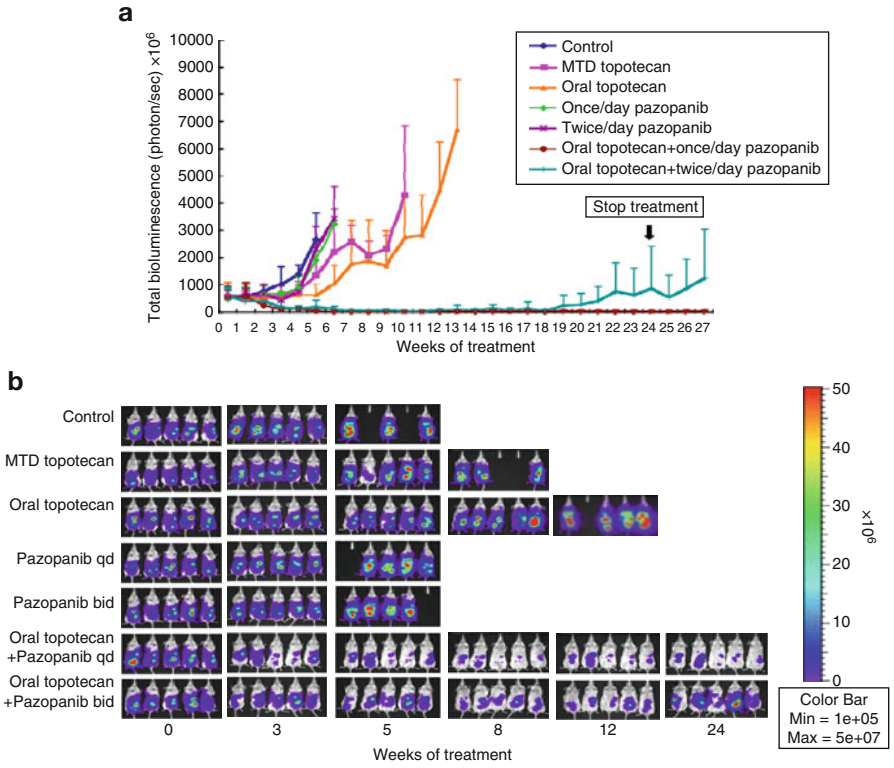

Fig. 1.2 Response assessed by bioluminescence: detection of relapsed/resistant disease. Detection of relapsing ovarian cancer xenografts after long-term response to nonstop daily therapy using the 25 mg/kg twice a day (b.i.d.) pazopanib plus oral metronomic topotecan protocol, detected by whole body bioluminescent imaging or photon release. (**a**) Impact of metronomic topotecan and pazopanib on ovarian cancer growth in mice. (**b**) Whole body optical imaging results (Taken from Hashimoto et al. [57])

therapy [50, 57]. Both in vitro and in vivo studies have shown that the resistant phenotype is expressed by the relapsing ovarian cancer cell population [50]. If the oral topotecan was acting solely through an antiangiogenic or a host-dependent immune-stimulating mechanism, the resistant phenotype should not be expressed by the relapsing tumor cell population.

Second, another reason to suggest that metronomic chemotherapy does not necessarily act primarily through an antiangiogenic or immune-stimulating type of mechanism, as opposed to operating through a direct tumor cell-targeting process, stems from other indirect observations. Thus, when a particular metronomic chemotherapy regimen is found to be highly effective when preclinically treating a certain type of cancer, e.g., breast cancer, it does not necessarily follow that that same regimen will show similar or even any efficacy when treating another type of cancer. An example of this is the doublet combination of metronomic oral cyclophosphamide with the oral 5-FU prodrug known as UFT (tegafur + uracil). This combination was found to cause remarkable prolongation of survival in mice with advanced metastatic human breast cancer [58, 59] despite having only modest effects on the growth of

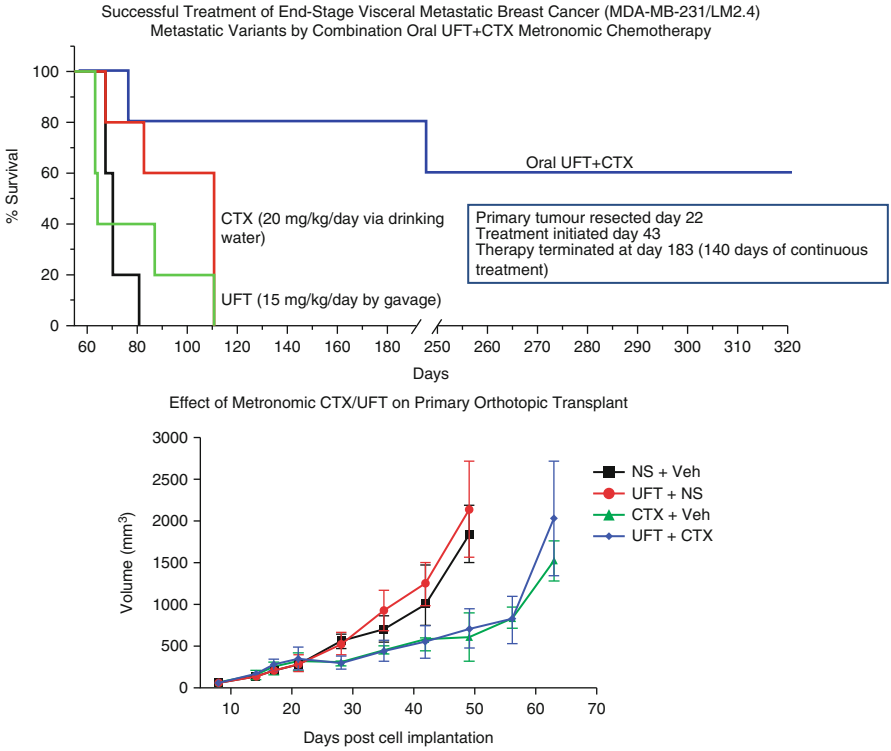

Fig. 1.3 Contrasting outcomes of metronomic chemotherapy using concurrent daily oral metronomic cyclophosphamide plus UFT to treat visceral metastatic disease in multiple organ sites established ~22 days after surgical resection of orthotopic primary tumors (LM2.4) (*upper panel*), a highly aggressive metastatic derivative of the MDA-MB-231 breast cancer cell line. The *lower panel* shows the effect of the same doublet therapy on the same line grown and treated as an orthotopic primary tumor, indicating much more modest effects and no effect of UFT alone or with cyclophosphamide. Note the duration of the successful therapy in the *upper panel* – 140 days of daily nonstop doublet metronomic chemotherapy. This experiment has been repeated several times and was pivotal to the decision to initiate a phase II trial in Italy (Dellapasqua et al. [71]) and which is now being evaluated in a randomized phase III trial in Switzerland based on the encouraging efficacy and safety results of the phase II trial (as described in the text) (Adapted from Munoz et al. [58])

established primary tumors (see Fig. 1.3). However, this same treatment combination has been tested by us in other preclinical model tumor systems, e.g., malignant melanoma [60], colorectal carcinoma [61], and ovarian cancer [57], and it was not found to possess significant antitumor activity (unpublished observations). Moreover, the potent antitumor effects of the UFT/cyclophosphamide metronomic chemotherapy combination in a setting of advanced metastatic disease are normally not achieved when treating advanced metastatic cancer with single agent antiangiogenic drugs such as bevacizumab, either preclinically, or clinically [62]. This would seem to preclude, or at least minimize, the possibility that the aforementioned metronomic chemotherapy protocol was acting only or mainly through an antiangiogenic mechanism. By way of example, in the same model of advanced metastatic breast cancer where the UFT/cyclophosphamide protocol seemed to be so effective [58], treatments

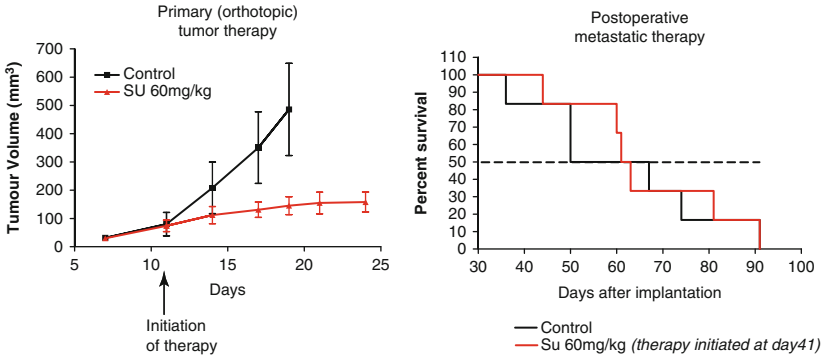

Fig. 1.4 Contrasting effects of daily sunitinib (*SU*) therapy in separate experiments on the growth of orthotopic primary breast cancers (*left graph*) vs. advanced metastatic disease, after the primary tumor was resected (at day 20) and therapy initiated at day 41 (*right graph*). The tumor line used for the study was a variant (called LM2-4) of the MDA-MB-231 human breast cancer line, isolated by Munoz et al. having aggressive spontaneous metastatic potential from established but resected primary tumors [58] (Taken from Guerin et al. [63])

instead with DC101, the anti-VEGFR-2 antibody, or either the TKIs pazopanib or sunitinib were all completely ineffective in prolonging survival [63]. An example of this (shown in Fig. 1.4) using sunitinib also shows that, in contrast, the same treatment is ineffective in mice with advanced metastatic disease. This would seem to correlate with the known finding that single agent bevacizumab (or sunitinib) is devoid of meaningful antitumor activity when treating advanced metastatic breast, or colorectal cancer, among other types of malignancy [62].

One intriguing possibility to account for the direct antitumor effects of metronomic chemotherapy is through an effect on the putative cancer stem cell (CSC) or tumor-initiating cell (TIC) subpopulation [64]. Indeed, there is evidence from a small number of published studies [65–67], beginning with our own [65], that CSCs may be sensitive to metronomic chemotherapy, in contrast to using the same chemotherapy drugs at MTDs, where such therapy may spare and thus enrich such cells [68]. This might also be consistent with the finding that metronomic chemotherapy protocols, unlike MTD chemotherapy, do not usually cause rapid tumor responses (i.e., marked reductions in tumor mass) but can nevertheless result in long-term disease control, at least preclinically [58, 69]. In theory, this is consistent with targeting the CSC subpopulation, as this would not be associated with a rapid and marked reduction in tumor mass but could still result in long-term antitumor effects and prolonged survival if indeed it is the case that CSCs represent the major tumor cell population for causing tumor regrowth/regeneration after therapy [64, 68]. Clearly, additional in-depth studies are needed to strengthen and validate the hypothesis that metronomic chemotherapy regimens may indeed target the CSC subpopulation, unlike MTD chemotherapy.

An important aspect highlighted by some of the aforementioned studies concerns the impact of metronomic chemotherapy when treating advanced metastatic disease – a daunting and challenging therapeutic circumstance in the clinic. When our

first paper on metronomic chemotherapy was published in 2000, not only did it spawn the editorial commentary by Hanahan et al. [1] but also others, including one published in *Nature Medicine* by Fidler and Ellis [70]. In the latter, these two authors mentioned a cautionary note about the ostensibly dramatic antitumor effects that we reported using the metronomic vinblastine plus DC101. This neuroblastoma model we used involved subcutaneously transplanted human tumor xenografts, and Fidler and Ellis noted that this could constitute yet another example of a seemingly spectacular antitumor treatment in mice that would never translate to the clinic based on using a faulty preclinical model that grossly exaggerates antitumor efficacy. Such models often involve subcutaneously (ectopic) transplanted tumor cell lines, instead of orthotopic primary tumor transplantations or treatment of advanced metastatic disease [70]. This editorial as well as later personal comments by Dr. Ellis mentioned to me at meetings stimulated a major research initiative in my lab, namely, to develop models of advanced metastatic disease that could be used to evaluate various therapeutic investigational treatments [59]. These included metronomic chemotherapy, antiangiogenic drugs, or combination of both, as illustrated in Figs. 1.1, 1.2, 1.3, and 1.4. Indeed, the results in Fig. 1.3 using UFT plus cyclophosphamide helped contribute to the decision to initiate a nonrandomized phase II trial evaluating daily oral metronomic capecitabine plus cyclophosphamide with bevacizumab for the first-line treatment of metastatic breast cancer patients [71]. The encouraging results of this small trial [71] resulted in the decision to initiate a limited randomized phase III trial in Switzerland investigating the same three-drug ("BEX") regimen (www.clinicaltrials.gov, clinical trial identifier # NCT01131195).

Our preclinical, and those of many others findings have contributed to the decision to initiate a number of other clinical trials that are underway or which have been completed evaluating metronomic chemotherapy in patients with metastatic disease – especially breast or colorectal cancer, as shown in Table 1.1. As mentioned earlier, the results of the first such randomized phase III trial known as CAIRO3 have been recently announced. This trial involved first-line treatment of metastatic colorectal cancer patients who received standard oral capecitabine plus oxaliplatin (CAPOX) therapy plus bevacizumab (CAPOX-B). The capecitabine was given in a 3-week cycle, i.e., every day for 2 weeks followed by a 1-week break. The daily dose was 1,000 mg/m^2 twice a day for this "upfront" therapy which lasted for about 4.5 months and consisted of six cycles of therapy. Historically, once this therapy is completed, patients do not normally receive any further treatment, they are simply observed, and when evidence of tumor progression/relapse occurs, the patients are either retreated with the same therapy or given another therapy. What was evaluated in CAIRO3 in patients who did not progress on the initial therapy was whether it would be possible to continue some sort of therapy (i.e., there would be no break) that would have an added benefit; this meant it would have to be minimally toxic and tolerable – and that is why it was decided to switch the upfront capecitabine protocol (with no oxaliplatin) to a less toxic "metronomic" daily nonstop schedule where the drug was given at 650 mg/m^2 twice a day, again with bevacizumab. Progression-free survival (PFS) between the two groups was evaluated, and once patients in both groups progressed, they were retreated with the initial

Table 1.1 Current randomized phase III trials of metronomic chemotherapy

Country and clinical trial identifier #	Details/title of trial
USA (NCT00925652)	Bevacizumab, metronomic chemotherapy (CM), diet, and exercise after preoperative chemotherapy for breast cancer (ABCDE)
China (NCT01112826)	Efficacy of capecitabine metronomic chemotherapy in triple-negative breast cancer (SYSCBC-001)
Switzerland (NCT01131195)	Bevacizumab and paclitaxel or bevacizumab, cyclophosphamide, and capecitabine as first-line therapy in treating women with locally advanced, recurrent, or metastatic breast cancer (SAKK 04/29)
Netherlands (NCT00442637)	Maintenance treatment vs. observation in advanced colorectal cancer (CAIRO3)
Sweden (NCT01229813)	Bevacizumab (and metronomic capecitabine) chemotherapy followed by K-ras randomization to maintenance treatment for first-line treatment of metastatic colorectal cancer (ACT2)

Adapted from Kerbel [85]
Listed in www.clinicaltrials.gov; another breast cancer (in hormone-nonresponsive disease) trial of adjuvant maintenance (1 year) daily metronomic oral cyclophosphamide plus methotrexate following postsurgical standard induction adjuvant chemotherapy has been completed, but the results not yet announced (www.ibcsg.org)

upfront "conventional" CAPOX-B regimen and PFS was again evaluated (called "PFS 2") which was the primary endpoint. In a sense, this is a version of the Hanahan concept of "chemo-switch" and also what we published in 2000 in our neuroblastoma model [3]. There was a marked statistically significant benefit in PFS1 and also PFS2 [4]. The chemo-switch regimen has also been evaluated clinically in other indications such as by Bellmunt et al. in renal cell carcinoma patients [72].

There are some complications about interpreting the trial result. For example, is it really the case that both the 'metronomic' maintenance capecitabine and maintenance bevacizumab are necessary to obtain the clinical benefits that were observed? That the capecitabine is indeed necessary stems from the fact that a similar first-line metastatic colorectal trial was undertaken by a Swiss group (SAKK) in which they evaluated maintenance *bevacizumab only* and it showed no benefit [73]. So a question which is raised is whether bevacizumab is necessary to use along with the maintenance capecitabine in the CAIRO3 colorectal trial. Based on preclinical findings, the likely answer is "yes." But obviously this question was not formally addressed in the CAIRO3 phase III trial design. Nonetheless, the results suggest that less toxic long-term metronomic chemotherapy regimens used as a maintenance therapy may be an ideal if not optimal circumstance for their application in the clinic.

1.2 Some of the Questions and Challenges That Lie Ahead for More Common Adoption of Metronomic Chemotherapy Concept

If one looks at the www.clinicaltrials.gov website under the heading of "metronomic chemotherapy," it has to be conceded that this treatment concept remains a niche therapeutic modality. Perhaps this might change with some additional phase

III clinical trial successes along the lines of CAIRO3. That it is a "niche" treatment concept is perhaps not surprising for a number of reasons. They relate to some of the disadvantages (or perception of disadvantages) associated with this treatment concept. First and foremost, there is the issue of how to define an optimal dose when using metronomic chemotherapy, i.e., doses that are below the MTD. By definition, this creates highly empirical situation. In this regard, it is perhaps reassuring that despite this empiricism, there have been some notable preliminary (tentative) phase II trial successes [71, 74, 75] which in part led to the later decision to initiate some phase III trials. Nevertheless, this remains a difficult issue to resolve. The results of CAIRO3 also raise a question about whether the terminology of "low dose" should be used synonymously with the term "metronomic chemotherapy." For example, in the CAIRO3 trial, the cumulative dose of maintenance capecitabine when given daily over a 3-week period was almost the same as when it was given more conventionally upfront every day for 2 weeks followed by a 1-week break [4]. In other words, the daily dose is less than the conventional MTD-type daily dose, but the cumulative dose is about the same. Perhaps this is the way we should think in the foreseeable future about how to dose chemotherapy drugs when given "metronomically," i.e., the total dose per unit time remains roughly the same as the MTD schedule but is given more frequently at appropriately lower doses. However, more work needs to be done to validate this hypothesis.

A second problem relates to the lack of defined clear-cut mechanisms to explain how metronomic chemotherapy actually works. This becomes ever more of a problem in an era where there is a need, if not an expectation, for knowing the precise molecular basis of a therapy and the development of molecularly targeted drugs. On the one hand, while it may be reassuring that metronomic chemotherapy may be due to multiple and convergent mechanisms, e.g., antiangiogenesis, immune stimulation, and targeting cancer cells directly including, perhaps, CSCs, the molecular mechanism by which they do so is largely unknown.

A third problem, in terms of gaining wider acceptance, ironically, is the reduced toxicity associated with metronomic chemotherapy compared to MTD chemotherapy. Toxicity to normal cells and tissues is often viewed as a surrogate for "antitumor activity," or efficacy, and thus lesser toxicity could mean reduced antitumor activity. Nevertheless, preclinical studies have shown that *long-term* metronomic chemotherapy regimens can be superior to the comparative effects of shorter-term MTD protocols, e.g., Hashimoto et al. [57] and du Manoir et al. [76].

These aforementioned problems can be tackled, at least to some extent by appropriately designed preclinical studies of the kind undertaken by a number of investigators such as Graciela Scharovsky [77–80] who are addressing such questions as defining promising or optimal sequencing and drug combinations, comparing MTD vs. metronomic chemotherapy head to head, examining cross-resistance between MTD and metronomic chemotherapy, among others.

With respect to additional advantages of metronomic chemotherapy, one that is becoming increasingly important, at least potentially, is cost, when using off-patent chemotherapy drugs and, in particular, oral agents that can be taken at home by patients. The rapidly and alarming escalating cost of new anticancer drugs is posing serious problems with respect to affordability even for countries

that have the financial resources to cope with such costs. But this cannot be sustained in all likelihood, and clearly for most cancer patients in low or middle income developing countries, the cost of new anticancer drugs is essentially an impossibility. Thus, there is a compelling need to evaluate whether metronomic chemotherapy regimens using less expensive off-patent and especially oral drugs really do have a therapeutic benefit in terms of prolonging survival [81]. From a profit incentive viewpoint, such regimens could be used in other countries such as the USA, Canada, western European nations, etc. in combination with new targeted therapies such as antiangiogenic agents, aromatase inhibitors [75], and oncogene-targeting drugs such as trastuzumab [82], among others. This could create an incentive for pharmaceutical companies to undertake appropriate (and expensive) combination phase III trials involving metronomic chemotherapy. Finally, with respect to safety and tolerability, the reduced toxicity associated with metronomic chemotherapy should be an advantage for elderly cancer patients, pediatric cancer patients, and perhaps also for undertaking adjuvant therapy in otherwise healthy patients who have minimal residual microscopic disease or possibly even no cancer at all. A case in point is the use of what might be called "metronomic chemotherapy in retrospect," namely, daily oral therapy with UFT over 2 years as an adjuvant treatment for non-small-cell lung cancer [83] or breast cancer [84]. It would be extremely interesting to evaluate such adjuvant chemotherapy protocols in conjunction with targeted agents in future clinical trials [85].

1.3 Summary

Over the last 15 years, the concept of metronomic chemotherapy has evolved from a descriptive preclinical phenomenon involving inhibition of angiogenesis as a primary or sole mechanism of action to a clinically validated treatment concept that is likely mediated by additional mechanisms of action. Current phase III clinical trials, when completed, should provide an indication of whether metronomic chemotherapy will remain a niche treatment concept or will assume much wider acceptance and application, so that it eventually becomes a significant component of mainstream medical oncology practice.

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Mechanisms of Action of Low-Dose Metronomic Chemotherapy

2

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Abstract

Evidence from a growing body of preclinical and clinical studies points to the efficacy of continuously administrating anticancer chemotherapeutic drugs in low doses. This relatively new treatment strategy concept is called low-dose metronomic (LDM) chemotherapy. The therapeutic efficacy of LDM has been assessed for reducing the tumor load during the acute phase and in delaying relapse during the maintenance phase. The major benefits found in using LDM include the lack of major toxicities or complications as compared to conventional chemotherapy regimens and improved quality of life. Traditional therapeutic modalities in oncology aim toward more specific tumor targets at the tumor microenvironment, whereas LDM chemotherapy acts on a broad spectrum of mechanisms, some of which are still not clear. We will discuss in this chapter several possible LDM chemotherapy anticancer mechanisms of action. Initially, LDM was considered an antiangiogenic treatment strategy; however, in the last decade additional preclinical studies uncovered other possible mechanisms including enhancing the antitumor immune response, substantially increasing the efficacy of targeted drugs by various mechanisms, targeting a subset of chemotherapy-resistant tumor cells, and blunting host response effects found following conventional therapy. While LDM chemotherapy is currently undergoing phase III clinical evaluation, its mechanisms of action are only partially understood. Elucidating LDM's mechanisms of action will give physicians an additional major weapon to deploy in the comprehensive management of cancer.

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2.1 Introduction

Initial studies which investigated the mechanism of action of low-dose metronomic (LDM) chemotherapy demonstrated that this treatment regimen solely acts by inhibiting tumor angiogenesis. Both Browder et al. and Klement et al. – the first two back-to-back studies introducing the concept of LDM chemotherapy – showed that low-dose cyclophosphamide (CTX) or vinblastine led to significant antitumor activity in Lewis lung carcinoma and neuroblastoma, respectively [1, 2]. Browder et al. also showed that the same tumors that responded to LDM were resistant to the conventional maximum tolerated dose (MTD) chemotherapy. Klement et al. demonstrated that the addition of an antiangiogenic drug, DC101, a VEGFR2-blocking antibody, to LDM vinblastine significantly and markedly improved LDM's therapeutic outcome in their neuroblastoma tumor model. While both studies suggested that LDM's mechanism of action is by blocking angiogenesis, this clearly does not explain why the addition of a VEGFR2-blocking antibody to LDM vinblastine significantly improved therapy outcome, unless additional complementary mechanisms are involved [2]. These and other results were the impetus for additional preclinical and clinical studies. In this review, we will focus on several possible mechanisms to explain the antitumor activities of LDM chemotherapy and their possible implications.

2.2 The Antiangiogenic Effects of Low-Dose Metronomic Chemotherapy

Tumor angiogenesis consists of a local division of endothelial cells from preexisting vessels, leading to neovasculature sprouting into the tumor. In addition, the systemic mobilization of bone marrow-derived proangiogenic cells, in particular endothelial progenitor cells (EPCs), incorporates into the tumor vessel wall, thus enhancing angiogenesis [3]. In the last several decades, efforts have been made to inhibit the formation of tumor blood vessels in order to halt tumor growth. Several antiangiogenic drugs have been approved by the Food and Drug Administration for the treatment of cancer. However, these therapies exhibit modest clinical benefits. In this context, LDM chemotherapy has also been identified as an antiangiogenic treatment strategy affecting various pathways of angiogenesis. LDM chemotherapy directly kills endothelial cells, induces natural inhibitors of angiogenesis, and inhibits systemic angiogenesis mediated by circulating endothelial precursor cells (CEPs). These various mechanisms of metronomic antiangiogenic effects are illustrated in Fig. 2.1 and are summarized below.

2.2.1 Low-Dose Metronomic Chemotherapy Directly Kills Endothelial Cells

The prolonged in vitro administration of low concentrations of cytotoxic drugs to rapidly dividing endothelial cells, such as human umbilical endothelial cells (HUVECs), induces cell apoptosis when compared to tumor cells which are more

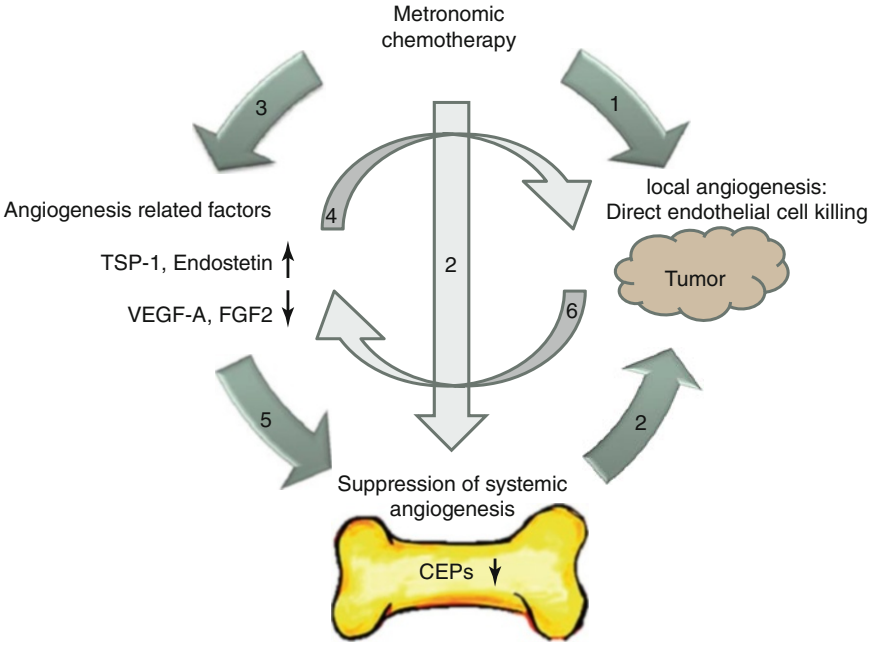

Fig. 2.1 An illustration of the antiangiogenic mechanisms of metronomic chemotherapy affecting tumor growth. Metronomic chemotherapy inhibits both (*1*) local angiogenesis by directly killing endothelial cells and (*2*) systemic angiogenesis by suppressing the levels of CEPs which then cannot home to the treated tumor. In addition, (*3*) metronomic chemotherapy reduces the expression of proangiogenic factors, e.g., VEGF-A and FGF2, and increases the expression of antiangiogenic factors, e.g., thrombospondin-1 (*TSP-1*) and endostatin. The changes in these factors can inhibit both (*4*) local and (*5*) systemic angiogeneses. (*6*) Tumor cells exposed to metronomic chemotherapy can also increase the expression of antiangiogenic factors, and as such they may support the antiangiogenic effect of this treatment regimen

resistant to such therapy [4]. This initial study has led to further testing various chemotherapy drugs administered in low doses to assess their antiangiogenic properties on endothelial cell viability. In another study, human microvascular endothelial cells (HMVECs) were exposed to L-OHP (an active metabolite of oxaliplatin), 5FU, and SN-38 (an active metabolite of irinotecan). The cells were cultured with these drugs in low doses continuously for 144 h. The results revealed that SN-38 but not 5FU or L-OHP inhibited endothelial cell proliferation. The combination of the three drugs, however, minimally affected colorectal cancer cell proliferation [5]. Taxanes and temozolomide (TMZ) have been also shown to act as antiangiogenic agents in continuous low-dose exposure in cultures [6, 7]. Murray et al. demonstrated that sorafenib, a small-molecule antiangiogenic drug blocking the tyrosine kinase of VEGF receptors, enhances the anti-endothelial cell effect when it was combined with continuous low doses of etoposide, paclitaxel, and TMZ in culture [8]. Additionally, mouse brain endothelial cells and U87 human glioblastoma but not C6 rat glioblastoma cells were shown to be sensitive to continuous low doses of

TMZ [7]. When this drug regimen was assessed in vivo, the authors demonstrated that LDM TMZ significantly reduced the antitumor activity in both C6 and U87 tumor-bearing mice, primarily due to its antiangiogenic activity as assessed by microvessel density [7]. These results further suggest that even when tumor cells are resistant to LDM TMZ in culture, they can be sensitive in the whole organism due to the drug's antiangiogenic effects.

The antiangiogenic activity of LDM chemotherapy has also been documented in vivo in several preclinical models. LDM doxifluridine suppresses tumor growth via its antiangiogenic activity, as assessed by an in vivo imaging technique. In this study, the addition of TNP-470, an antiangiogenic agent, to the LDM doxifluridine regimen resulted in a significant reduction of the growth of uterine carcinosarcoma in bearing mice compared to mice treated with each of the drugs and to the drug regimen alone [9]. This study also discusses additional results from various trials demonstrating the additional or even synergistic antitumor effects of LDM chemotherapy when combined with antiangiogenic drugs. For example, LDM topotecan in combination with pazopanib, a small-molecule inhibitor of angiogenesis, resulted in a significant increase in antitumor activity, due in part to reduced tumor microvessel density. The antiangiogenic effects seen in this study were related to the inhibition of systemic angiogenesis rather than inhibiting local antiangiogenic activity in treated tumors. However, no experiments were performed to test whether LDM topotecan may directly kill endothelial cells in culture [10]. Taken together, the aforementioned in vitro and in vivo preclinical studies suggest that continuous exposure of chemotherapy in low doses in the absence or presence of direct antiangiogenic agents markedly induces endothelial cell apoptosis in vitro and as a result increases their antitumor efficacy in vivo. Thus, one of the mechanisms of LDM chemotherapy is in inducing endothelial cell apoptosis, since in many cases these cells are more sensitive to the drug regimen than the tumor cells.

2.2.2 Low-Dose Metronomic Chemotherapy Alters the Expression of Angiogenesis-Related Factors

A balance between proangiogenic and antiangiogenic factors exists in tissues in order to maintain angiogenic homeostasis. In tumors this balance is violated as tumor cells secrete proangiogenic factors leading to increased endothelial cell sprouting and tumor angiogenesis [11]. Several studies investigated the antiangiogenic effects of LDM chemotherapy focusing on changes in the balance between angiogenic stimuli and natural inhibitors of angiogenesis. LDM CTX upregulates the expression of thrombostpondin-1 (TSP-1), a natural inhibitor of angiogenesis [12], in the serum of Lewis lung carcinoma-bearing mice. While tumor-bearing mice treated with LDM CTX express high levels of TSP-1 and subsequently exhibit enhanced treatment outcome, mice lacking the expression of TSP-1 (TSP1−/− mice) bearing Lewis lung carcinoma treated with the same therapy did not show any antitumor activity of such therapy [13]. In support of this study, it was shown that TSP-1 expression is upregulated in tumor cells and perivascular cells following LDM

CTX, indicating again the antiangiogenic effects of LDM CTX not related to direct endothelial cell killing [14].

In contrast, a combination of LDM CTX with high-dose doxorubicin exhibited a dramatic decrease in the expression of TSP-1 in the plasma of rats bearing prostate tumors, suggesting that the antiangiogenic effects mediated by the upregulation of endogenous inhibitors of angiogenesis can be negated when such treatment is combined with a bolus administration of other chemotherapy drugs [15]. Apart from TSP-1, other pro- and antiangiogenic factors are altered in response to LDM chemotherapy. It was recently demonstrated that LDM etoposide alters the angiogenic switch in tumors by inhibiting VEGF-A and FGF2 secreted from tumor cells and by increasing plasma levels of endostatin, a natural endogenous angiogenesis inhibitor [16]. Clinical studies showed decreased levels of angiogenic factors such as VEGF and PDGF-BB in cancer patients treated with LDM capecitabine or LDM CTX, methotrexate, and thalidomide [17, 18]. In addition, TSP-1 serum levels were upregulated in cancer patients treated with LDM CTX. However, the elevated levels of TSP-1 did not correlate with clinical benefits [19]. In patients with non-small lung cancer, levels of VEGF, VEGFR1, and TSP-1 were tested in the serum following either MTD or LDM cisplatinum and docetaxel chemotherapies. While MTD combined therapy induced a significant change in VEGFR1 and TSP-1 serum levels, the impact of LDM chemotherapy (using weekly docetaxel and cisplatinum regimen) did not alter these factors in the serum of treated patients. Surprisingly, MTD chemotherapy induced a significant long-lasting increase in TSP-1 levels and a decrease in VEGFR1 levels as opposed to LDM chemotherapy. The authors concluded that continued administration of LDM chemotherapy does not necessarily act as an antiangiogenic chemotherapy regimen when compared to MTD regimen [20].

2.2.3 Low-Dose Metronomic Chemotherapy Blocks Systemic Angiogenesis

While the effect of local angiogenesis is well established in cancer, the contribution of systemic angiogenesis (also called vasculogenesis) is greatly debated [21, 22]. Recent studies have shown that following acute therapy, systemic cells significantly contribute to the regeneration of neo-angiogenesis in treated tumors, thereby promoting tumor regrowth [23]. LDM chemotherapy was shown to substantially suppress the number of circulating bone marrow-derived proangiogenic cells (BMDCs) such as CEPs. The initial study in this direction tested levels of CEPs in lymphoma-bearing mice that underwent LDM or MTD CTX. While the MTD regimen induced a substantial increase in the number of CEPs in the blood, the LDM regimen significantly and continuously suppressed it. Once the LDM CTX therapy was terminated, the number of CEPs subsequently rose in peripheral blood followed by tumor regrowth [24]. These results further suggest that LDM CTX can suppress systemic angiogenesis mediated by CEPs. Based on this study, antiangiogenic drugs or treatment strategies thought to inhibit systemic angiogenesis have been further tested.

Mice treated with antiangiogenic drugs or with LDM chemotherapy using CTX, vinblastine, cisplatinum, or vinorelbine revealed that the maximal suppression in CEP levels in mice undergoing such therapy correlated with the maximum antiangiogenic activity [25, 26]. Therefore, the CEP suppression level could serve as a biomarker for the optimal angiogenic activity of both antiangiogenic drugs and LDM chemotherapy [25, 26]. More recent studies focused on CEP level measurements in mice treated with drug combinations involving an LDM chemotherapy regimen. For example, the administration of LDM taxanes such as docetaxel alone or in combination with AEE788, a dual EGFR and VEGFR inhibitor, resulted in a marked decrease in CEP levels in mice bearing ovarian cancers which led to a significant reduction in tumor growth and prolonged survival [6]. In another study, oral topotecan in LDM regimen in combination with pazopanib resulted in a marked reduction in viable CEPs as well as circulating endothelial cells (CECs) and reduced tumor microvessel density in several pediatric solid tumors [10]. Importantly, suppressed levels of CEPs were also documented in a drug combination in which acute therapy can sometimes induce rapid mobilization of CEPs. For example, studies conducted on mice treated with vascular disrupting agents (VDAs) revealed a marked and rapid elevation in CEP levels in the peripheral blood of treated mice [27]. The same effects have also been demonstrated in cancer patients enrolled in a phase I clinical study testing the anti-vascular agent AVE8062 [28]. Consequently, Daenen et al. reasoned that the combination of LDM CTX with VDAs may block the rapid mobilization of CEPs found following VDA therapy. They tested this by using mice bearing metastatic breast carcinoma or melanoma xenografts which were treated with OXi-4503, LDM CTX, or the combination of the two drugs. They found that levels of CEPs which were rapidly elevated following VDA therapy were significantly inhibited when such therapy was combined with LDM CTX. These anti-vasculogenic effects resulted in less colonization of BMDCs at the treated tumor, which is often seen following VDA therapy. The authors concluded that the combination of VDA and LDM CTX resulted in prolonged tumor control, in part due to the anti-vasculogenic activity of the metronomic chemotherapy [29].

Clinically, CEC and CEP levels were evaluated in cancer patients undergoing LDM chemotherapy to assess their prognostic or predictive value following antiangiogenic therapy. Mancuso et al. analyzed the kinetics and viability of CECs in advanced breast cancer patients treated with methotrexate, thalidomide, and LDM CTX. They found that increased levels of apoptotic CECs correlated with therapy outcome, suggesting that CECs may predict clinical response to metronomic/antiangiogenic therapy [30]. In another study, long-term interferon-α, thalidomide, and celecoxib treatment combination was tested in patients with slow-growing solid tumors. The levels of CEPs were analyzed during the course of the therapy, and the results suggest that low baseline levels of CEPs predict subsequent clinical benefits [31]. Another recent study strongly supports these findings. Investigators demonstrated that high CEP levels in hepatocellular cancer patients treated with sorafenib and LDM tegafur and uracil were associated with poor survival [32]. CEC levels, on the other hand, were evaluated in breast cancer patients treated with LDM CTX, capecitabine, and bevacizumab (an

anti-VEGF-A neutralizing antibody). As opposed to CEPs, high baseline levels of CECs predicted prolonged clinical benefits. It was suggested that active vascular turnover in tumors may result in high baseline levels of CECs which then can effectively be blocked by an antiangiogenic drug or treatment regimen [33].

Not all chemotherapy drugs administered in an LDM regimen may affect systemic angiogenesis. A recent study by Francia et al. has demonstrated that oral gemcitabine administered daily inhibits tumor growth and angiogenesis, but does not significantly suppress the levels of CEPs. Therefore, this drug regimen has antitumor and antiangiogenic activity without inhibiting the systemic angiogenesis as seen with other LDM chemotherapies [34]. Overall, these studies highlight the impact that most chemotherapy drugs administered in an LDM chemotherapy regimen have on BMDC levels, particularly on CEPs and CECs. However, LDM's effects on other BMDCs known to contribute to systemic angiogenesis, e.g., hemangiocytes [35] and myeloid-derived suppressor cells [36], still need further investigation.

2.3 Additional Antitumor Activity Mechanisms of Low-Dose Metronomic Chemotherapy

In the last decade, new mechanisms of antitumor activity of LDM chemotherapy besides those related to antiangiogenic activity have been proposed and investigated. It has been shown that LDM chemotherapy can enhance the immune response against tumor cells thereby promoting tumor growth control in an "immunotherapy-like" strategy. This mechanism will extensively be covered in another chapter in this book. Additionally, limited evidence exists regarding the potential of LDM chemotherapy in targeting cancer stem cells (CSCs), a subpopulation of tumor cells with stem cell characteristics that are normally resistant to conventional therapy. Lastly, LDM chemotherapy blocks host effects promoting tumor regrowth commonly found following acute therapy [37]. Table 2.1 presents a summary of several proposed mechanisms of action for the antitumor activity of LDM chemotherapy. These proposed mechanisms are discussed in more detail below.

2.3.1 Low-Dose Metronomic Chemotherapy Enhances the Antitumor Activity of Oncolytic Virotherapy

Oncolytic virotherapy is one of the recent novel investigated routes of cancer therapy which has entered clinical testing. The efficacy of oncolytic virotherapy combines the ability of the virus to directly destroy cancer cells on one hand and to increase the immune system's response against cancer cells on the other [38]. The current oncolytic viruses are still under thorough investigation both preclinically and clinically. Although they are considered nonpathogenic to humans, they were found to selectively replicate in human cancer cells, thereby promoting cancer regression [39]. One of the complications of oncolytic virotherapy is the immune system's

Table 2.1 Additional mechanisms of action for LDM chemotherapy

Item	The effect of LDM chemotherapy	The effect of MTD chemotherapy	Course of action
Cancer stem cells	LDM chemotherapy in combination with antiangiogenic therapy might reduce the number of CSCs	CSCs are resistant to conventional chemotherapy and radiotherapy	Angiogenic factors and blood vessels support CSCs Some CSCs are proangiogenic and/or VEGF dependent
Host response to therapy	LDM chemotherapy may blunt the host response effects seen following conventional therapy	Tumor regrowth and metastasis acceleration are sometimes found following MTD chemotherapy	LDM chemotherapy regimen, as opposed to MTD regimen, induces antiangiogenic effects in part by suppressing the levels of CEPs in peripheral blood. MTD chemotherapy promotes BMDC mobilization and tumor homing by the upregulation of G-CSF and SDF-1
Oncolytic virotherapy	Improved sensitivity of tumor cells to the virotherapy by LDM chemotherapy	Tumor regrowth delay is observed following MTD chemotherapy in combination with virotherapy	The combination of LDM chemotherapy with oncolytic virotherapy increases the viral activity by suppressing several immune cell types normally acting against the virus

In addition to the antiangiogenic activity of LDM chemotherapy, some additional mechanisms of action were proposed. However, they are still undergoing thorough investigation, and limited evidence exists to support their course of action. LDM chemotherapy can (a) eliminate cancer stem cells, (b) blunt host response effects to conventional therapy, and (c) synergize with oncolytic virotherapy. Cancer stem cells (CSCs), granulocyte colony-stimulating factor (G-CSF), stromal-derived factor 1 (SDF-1), and circulating endothelial precursor cells (CEPs)

reaction against the viral infection. Therefore, the use of LDM chemotherapy which can suppress or deplete several immune cell types that normally act against the injected virus is considered a therapeutic advantage when combined with oncolytic virotherapy [40]. This treatment combination was found to be efficacious in several preclinical tumor models, such as B16 melanoma [40], ovarian carcinoma [41], glioblastoma [42], and pancreatic cancer [43] among others. In addition to the impact of LDM chemotherapy on the immune system, other effects may exist. For example, LDM paclitaxel in combination with an oncolytic virus in relapsed ovarian cancer resulted in substantial treatment benefits. The reason was that paclitaxel therapy promoted a morphological change in replicating tumor cells, which in turn induced an immune response against the tumor cells leading to an induction of the immune system against the tumor cells, especially those which were already infected with the oncolytic virus [41]. Clinically, patients with advanced solid tumors who progressed after conventional therapies were treated with a combination of LDM CTX and oncolytic adenovirus therapy. While the purpose of LDM CTX was to eliminate the T-regulatory cell activity, the intra-tumoral injection of the oncolytic virus increased cytotoxic T cells and induced Th1-type immunity in

those patients, leading to a decrease in tumor burden. These results further suggest that LDM chemotherapy promotes immunological effects which can enhance the oncolytic virotherapy in several ways [44]. CTX in low doses is not the only drug that can act synergistically with oncolytic virotherapy; LDM TMZ can also enhance the antitumor activity of oncolytic virotherapy in patients with refractory tumors. This treatment regimen inhibits regulatory T-cell activity, which in combination with oncolytic adenovirus therapy results in tumor responses in two thirds of the patients. The authors suggest that LDM chemotherapy promotes tumor cell autophagy and elicits antitumor immune responses which results in improved oncolytic virus therapy efficacy [45]. It should be noted that oncolytic virotherapy has been tested also with MTD chemotherapy and found to be superior in terms of treatment efficacy compared to conventional MTD monotherapy [46]. In addition, tumor regrowth observed following MTD chemotherapy can be significantly delayed with treatment involving oncolytic virotherapy [47]. However, most of these studies are still under thorough clinical evaluation in early phase studies, and the mechanisms of action of these treatment combinations are not fully understood. Overall, the combination of LDM chemotherapy with oncolytic virotherapy enhances the activity of the virus against the tumor cells by altering the immune system against the virus and/or by improving the sensitivity of the tumor cells to the virotherapy.

2.3.2 Low-Dose Metronomic Chemotherapy Prevents Host Effects Seen in Response to Acute Therapy

Rebound angiogenesis has often been seen following treatment with MTD chemotherapy, in part due to a rapid mobilization and tumor homing of systemic angiogenic cells, e.g., CEPs to the treated tumor site, leading to tumor regrowth [3]. As opposed to MTD, LDM chemotherapy induces its antiangiogenic effects in part by suppressing the levels of CEPs in the blood [25]. Importantly, it has been suggested that the changes in the levels of CEPs in response to acute therapy are not associated with the tumor type or tumor stage but rather almost entirely related to the response of the host which generates such effects, especially since some of the experiments were performed on non-tumor-bearing mice [3, 27, 48].

We have recently shown that following MTD therapy, a rapid and significant upregulation of host G-CSF and SDF-1 was observed in the plasma of treated mice and cancer patients [3]. These factors are known to accelerate BMDC mobilization and homing into tumors; therefore, they could explain the regrowth of tumors following acute therapy [3, 49]. Additionally, the host effect in response to acute therapy is not limited to boosting tumor angiogenesis, but it may also accelerate metastasis spread [50]. These pro-tumorigenic and pro-metastatic effects found following MTD chemotherapy were also reported after other therapies including small-molecule antiangiogenic drugs [51] and VDAs [49]. There is some evidence that LDM chemotherapy can negate these host proangiogenic and pro-tumorigenic effects. The administration of LDM regimen following an acute dose of chemotherapy markedly improved the treatment outcome of pancreatic, breast, and prostate

cancers as well as erythroleukemia [52, 53]. Vives et al. recently demonstrated that LDM CTX or gemcitabine administered following acute MTD therapy of the same drug was superior in terms of antitumor activity associated with decreased angiogenesis and reduced metastasis for the treatment of ovarian and pancreatic adenocarcinoma models when compared to any of the treatments involving monotherapy regimen. In fact, peritoneal metastases were documented only in the control and MTD treatment groups, but were absent in the group consisting of MTD and LDM chemotherapy drug combination [54]. Hanahan and colleagues termed this combined regimen as a "chemo-switch" in which MTD chemotherapy (either alone or in combination with targeted agents) is followed by LDM maintenance therapy [52]. In the clinic, the combination of MTD and LDM therapy was recently tested in a multi-targeted chemo-switch regimen using sorafenib, gemcitabine, and LDM capecitabine for the treatment of metastatic renal cell cancer. The authors reported that the response rates of the combined therapy were greater than what was documented for gemcitabine and capecitabine or sorafenib monotherapy. These initial findings suggest a synergistic activity of the chemo-switch concept that needs further clinical evaluation [55].

Although LDM on its own leads to antiangiogenic effects, a remarkable synergistic antitumor effect was observed when an LDM chemotherapy regimen was combined with an antiangiogenic drug or with a VDA (for review see [56, 57]). As mentioned above, the combination of a VDA with continuous administration of LDM CTX resulted in decreased tumor regrowth compared with VDA monotherapy, due in part to the inhibition of acute CEP mobilization found following VDA monotherapy [29]. In addition, LDM topotecan administered in combination with pazopanib [58] showed significant improvement in overall survival of mice bearing metastatic ovarian cancer [59, 60]. The superior effects of the maintenance LDM chemotherapy which was administered after acute therapy could be explained by the fact that LDM regimen reduces the systemic involvement of BMDCs that are rapidly mobilized following some acute therapies as demonstrated by Daenen et al. [29]. In addition, LDM regimen reduces the expression levels of several circulating proangiogenic factors induced in response to the targeted therapy [54, 59, 60]. Therefore, blocking the pro-tumorigenic activities generated by the host in response to acute therapy explains the treatment superiority of combining a targeted therapy with LDM chemotherapy, even when the same drug is used in both regimens.

2.3.3 Low-Dose Metronomic Chemotherapy May Disrupt the Cancer Stem Cell's Niche

A subpopulation of cells in the tumor mass has recently been characterized as tumor "stem cells" since these cells can initiate tumor growth and metastasis. Such cells are termed cancer stem cells (CSCs) or tumor-initiating cells (TICs) [61]. The properties of CSCs are quite similar to those of normal stem cells. CSCs have the ability to initiate tumor growth, drive tumor cell proliferation, and differentiate into multilineage cells and to contain a self-renewal capacity [61]. Recent studies showed that

CSCs possess a strong DNA repair system, which distinguishes them from other "more differentiated" tumor cells [62]. Like stem cells, CSCs resist many conventional therapies including chemotherapy and radiation. As such, they are probably the sole viable subpopulation of tumor cells left after therapy. Ongoing research into new treatment modalities which can kill CSCs are currently being undertaken [63]. In terms of angiogenesis, a growing body of evidence suggests that CSCs require angiogenic factors and blood vessels to maintain their characteristics. CSCs were found to reside in close proximity to tumor vasculature [64, 65]. Disrupting the VEGF-neuropilin axis was found to decrease the number of CSCs, suggesting that CSCs are angiogenic or VEGF dependent [65, 66]. CSCs of C6 rat gliomas secrete both VEGF and SDF-1 used to promote systemic and local angiogenesis thereby contributing to tumor growth [67]. Therefore, antiangiogenic therapy, in particular anti-VEGF therapy, was predicted to possibly eradicate CSCs. Indeed, in several preclinical studies it was demonstrated that anti-VEGF therapy reduces the number of CSCs in treated tumors thereby explaining the increased treatment efficacy of chemotherapy in combination with antiangiogenic therapy [65, 66]. For other antiangiogenic treatment strategies, such as LDM chemotherapy, only limited literature exists. Treatment of C6 rat glioma-bearing mice with LDM CTX alone or in combination with an antiangiogenic drug (DC101) led to a reduced number of sphere-forming tumor cells that are usually enriched with CSCs [23]. In a hepatocellular carcinoma model, the combination of LDM CTX with an antiangiogenic drug led to tumor dormancy as long as the LDM chemotherapy regimen was maintained. However, once the mice were removed from the maintenance treatment protocol, tumor regrowth was subsequently observed. Although the authors focused on tumor dormancy, others suggested that dormant tumor cells could serve as CSCs since they can initiate tumor growth [68]. Interestingly, in another study in which MTD chemotherapy was followed by maintenance LDM therapy, the authors documented that the combination of chemo-switch therapy resulted in a decreased number of CSCs in both pancreatic and ovarian cancers using CD133, CD44, and CD24 as markers, which are selectively expressed on CSCs of such tumor types [54]. Overall, while limited evidence suggests that LDM chemotherapy may affect the viability and number of CSCs, more research is required to elucidate the mechanism by which LDM chemotherapy acts against CSCs.

2.4 Summary

Efforts to uncover the mechanisms of action of LDM chemotherapy are still ongoing. Several mechanisms have been presented which can explain the antitumor activity of this treatment modality. However, LDM's mechanisms of action are only partially understood, and we are far from comprehending the complete picture. Some of the benefits of using LDM chemotherapy in the clinic are the following: the usually low costs of such drugs, improved quality of life of treated patients, and the lack of major toxicities and clinical complications [69]. As such, LDM chemotherapy can be offered as an alternative treatment for conventional therapy. It can be

given during the acute phase to reduce tumors and in times of remission as a maintenance therapy to delay relapse and as a palliative treatment for advanced incurable metastatic diseases [37, 56]. Recent clinical and preclinical studies demonstrate (mostly empirically) that LDM chemotherapy substantially improves the antitumor activity of other anticancer drugs such as antiangiogenic small-molecule drugs [70]. While the combination of small-molecule drugs along with MTD chemotherapy regimen usually resulted in major toxicities and complications, LDM chemotherapy as a replacement has been shown preclinically to work well. It should be noted that the results of several phase III clinical studies utilizing LDM chemotherapy regimen in combination with other targeted drugs will soon be announced. This may lead to a paradigm shift in the way we treat cancer [37, 56]. Meanwhile, the lack of a thorough understanding on how LDM chemotherapy acts against tumor cells and the empirical nature of its evaluation in the clinic probably moderate the enthusiasm among clinicians in extensively using this treatment modality for cancer [37, 69]. Further experimentation toward elucidating LDM chemotherapy's mechanisms of action will pave the way for the intelligent use of this treatment regimen benefiting cancer patients worldwide.

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Effects of Metronomic Chemotherapy on Immunity

3

Mamoru Harada

Abstract

Chemotherapeutic agents are widely used for cancer treatment. However, these agents have the potential to kill tumor cells as well as nontumor cells, including immune cells. Therefore, conventional and maximum-tolerated dose chemotherapy is inevitably associated with the risk of immunity deterioration. Metronomic chemotherapy is a unique protocol that administers chemotherapeutic agents at relatively low doses, without prolonged drug-free periods. Metronomic chemotherapy was primarily developed to target circulating endothelial progenitor cells to inhibit tumor angiogenesis. Alternatively, certain chemotherapeutic agents have immunostimulatory effects. Specifically, cyclophosphamide (CTX) and gemcitabine (GEM) administration can decrease two major immunosuppressive cells, regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs), respectively, both of which increase in tumor-bearing hosts. However, administration protocols heavily influence the host's immunity because chemotherapeutic agents potentially kill proliferating lymphocytes. To this end, we investigated the effects of a CTX administration protocol on the in vivo induction of antitumor T cells in a preclinical model. We found that CTX administration at 4-day intervals deteriorated antitumor T cell immunity. Given these findings, we further tested a combination chemotherapy protocol with CTX and GEM at 8-day intervals and found that without impairing immunological competence, this protocol elicited antitumor T cells by decreasing Treg cells and MDSCs. In this chapter, I outline the in vivo induction of antitumor T cell immunity after chemotherapy, review the effects of metronomic chemotherapy on immunity, and discuss its underlying mechanisms.

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3.1 Introduction

Chemotherapy is the most frequent treatment modality for cancer patients. In general, its in vivo antitumor effects are dose dependent, and the clinically admissible dosage is the maximum dose at which patients can tolerate the adverse effects. When chemotherapeutic agents are administered to cancer patients at high doses, remarkable tumor regression can be induced; however, myelosuppressive side effects and immunosuppression are inevitable. Alternatively, recent reports suggest that antitumor immunity plays a crucial role in controlling tumor growth after chemotherapy. Chemotherapy can induce cell death in a portion of tumors, subsequently promoting dendritic cells (DCs) to uptake tumor antigens that are released from dying tumor cells and priming tumor-reactive T cells. Thereafter, these T cells exhibit cytotoxicity against tumor cells. Therefore, chemotherapy protocols should be carefully designed to not impair the immunological competence of tumor-bearing hosts.

Metronomic chemotherapy refers to the administration of chemotherapeutic agents at relatively low, minimally toxic doses, without prolonged drug-free periods. This type of chemotherapy has been suggested to be more effective and to have fewer toxic side effects compared to conventional, maximum-tolerated dose chemotherapy [1–4]. In fact, metronomic chemotherapy has been used in patients with several types of cancer, and clinical responses have been observed [5–7]. Metronomic chemotherapy primarily targets circulating endothelial progenitor cells and inhibits angiogenesis via production of thrombospondin-1 [8, 9]. However, some preclinical and clinical studies suggest that antitumor immunity is involved in antitumor effects after metronomic chemotherapy. In this chapter, I outline antitumor immunity after chemotherapy and review the effects of metronomic chemotherapy on immunity. I also discuss the underlying mechanisms and the optimal chemotherapy protocols that enhance antitumor immunity.

3.2 The Importance of Antitumor Immunity in Controlling Tumor Growth After Chemotherapy

Recent reports revealed that host immunity plays an important role in controlling tumor growth after chemotherapy [10]. As shown in Fig. 3.1, chemotherapeutic agents kill a portion of the tumor, and dying tumor cells release tumor antigens in the microenvironment. Tumor-infiltrating DCs uptake these antigens and migrate to draining lymph nodes (LNs), which are sites that elicit adaptive immunity in vivo. DCs present tumor antigen-derived peptides to $CD4^+$ T helper cells in the context of MHC class II molecules. Thereafter, they present tumor antigen-derived peptides to $CD8^+$ cytotoxic T cells in the context of MHC class I molecules. Subsequently, primed T cells migrate to the tumor site and exhibit cytotoxicity against tumor cells. When the immunological competence of tumor-bearing hosts is impaired, T cells fail to affect tumor cells, thereby allowing the tumor to grow.

The Zitvogel and Kroemer laboratories have revealed detailed mechanisms by which antitumor T cell immunity can be induced after chemotherapy [10, 11]. The presentation of tumor-derived antigens by DCs to T cells is a critical step in the in vivo elicitation

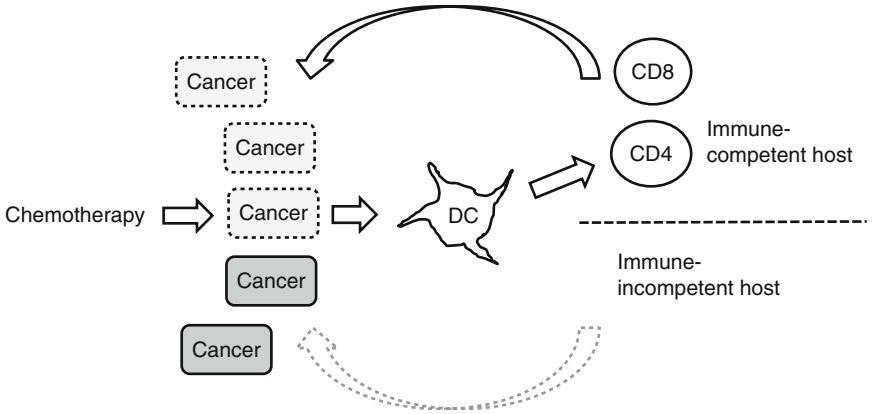

Fig. 3.1 Importance of host immunity in controlling tumor growth after chemotherapy

of antitumor T cell immunity, and some chemotherapeutic agents such as anthracycline are known to exploit this process. In anthracycline-treated dying tumor cells, calreticulin, which is constitutively expressed in the endoplasmic reticulum, migrates to the cell surface, provides phagocytic signals to DCs, and consequently promotes their uptake [12]. Simultaneously, dying tumor cells secrete high-mobility-group box 1 protein as a "danger" signal to DCs, resulting in efficient processing and cross-presentation of tumor antigens by DCs [13]. These studies further revealed that dying cancer cells release ATP and stimulate purinergic receptor on DCs, leading to inflammasome formation and the subsequent release of interleukin (IL)-1β [14]. Thereafter, DCs move to draining LNs and prime tumor antigen-specific $CD4^+$ T cells and, subsequently, $CD8^+$ T cells. This type of "immunogenic" tumor cell death is crucial for treatment-associated prognosis and for the survival of tumor-bearing hosts. Importantly, these phenomena are not limited to murine models; they are also seen in breast cancer patients [13, 14]. These accumulating data underscore the idea that antitumor immunity plays an important role in controlling tumor growth after chemotherapy.

3.3 Immunosuppressive Cells in Tumor-Bearing Hosts

Recent advances in tumor immunology have identified many tumor antigens that can be recognized by T cells, and laborious studies using clinical samples revealed the existence of tumor-specific T cells in certain cancer patients [15]. Additionally, it has been widely accepted that antitumor T cells are the most potent effector cells against tumor cells. However, there are several barriers to inhibiting the antitumor T cell response in tumor-bearing hosts. Specifically, the tumor-bearing state is usually associated with immunosuppression by immune-suppressive cells, including $CD4^+$ $CD25^+$ regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs) [16, 17]. Treg cells show immunosuppressive activity via immunosuppressive cytokines and cell-contact mechanisms. Their presence at tumor sites correlates with an unfavorable prognosis [18, 19]. MDSCs consist of two subpopulations: monocytic

MDSCs and granulocytic MDSCs [20]. MDSCs increase in tumor-bearing hosts, likely as a result of chronic inflammation, and inhibit T cell responses in cancer patients [21, 22]. For successful induction of the antitumor T cell response, immunosuppression mediated by these cells must be overcome. Although several antibodies and reagents can decrease Treg cells or MDSCs [23–28], effective and clinically applicable methods or protocols that decrease or ablate these two immunosuppressive populations in tumor-bearing hosts have not yet been established.

3.4 Effect of Chemotherapeutic Agents on Immune Cells

Several chemotherapeutic agents have the potential to stimulate antitumor immunity through the mitigation of immunosuppression by Treg cells and MDSCs and/or stimulation of immune cells.

3.4.1 Treg Cells

Cyclophosphamide (CTX) is a representative agent with immunostimulatory potential. Many studies have shown that low-dose CTX can increase antitumor immune responses in tumor-bearing hosts by mitigating Treg cell-mediated immunosuppression [4, 29, 30]. In a rat colon carcinoma model, Ghiringhelli et al. reported that the single administration of CTX depleted Treg cells, delayed tumor growth, and cured tumor-bearing hosts when followed by immunotherapy [31]. Low-dose CTX was revealed to decrease the cell number and inhibit the suppressive capability of Treg cells [32]. In addition, it was shown that the single administration of low-dose CTX augmented the antitumor immune responses of DC vaccines by reducing the proportion of Treg cells in tumor-bearing mice [33]. Roux et al. used murine models to show that Treg cells inhibited the ability of tumor-infiltrating DCs to mediate tumor necrosis factor-related apoptosis-inducing ligand-induced tumor cell death and that the depletion of Treg cells by CTX eradicated tumors [34]. Furthermore, Wada et al. used an autochthonous prostate cancer model and reported that CTX augmented the antitumor immune response [35]. This effect is associated with the transient depletion of Treg cells in tumor-draining LNs, but not in the peripheral circulation. We also reported that low-dose CTX relieved $CD4^+$ Treg-mediated immunosuppression and restored T cell proliferation and interferon (IFN)-γ production in murine colon tumor-bearing mice [36].

How can CTX deplete Treg cells or mitigate their immunosuppressive function? One plausible explanation is that CTX kills proliferating cells. It may be that Treg cells increase their proliferation capacity in tumor-bearing hosts. Interestingly, Zhao et al. revealed that low levels of ATP in Treg cells attenuated glutathione synthesis, leading to decreased CTX detoxification and increased sensitivity of Treg cells to low-dose CTX [37]. In addition, it was reported that the immunostimulatory effects of low-dose CTX are controlled by inducible nitric oxide synthase [38].

In addition to CTX, low-dose gemcitabine (GEM) was reported to deplete Treg cells and to improve the survival of pancreatic tumor-bearing mice [39].

3.4.2 MDSCs

It is widely accepted that MDSCs play a crucial role in tumor-associated immunosuppression [21, 22]. MDSCs are immature myeloid cells that do not differentiate into mature dendritic cells, granulocytes, or macrophages. This population exerts immunosuppressive effects on antitumor T cells through arginase-1, reactive oxygen species, IL-6, and IL-10. Several chemotherapeutic drugs were reported to decrease MDSCs. Suzuki et al. reported that GEM decreased the number of MDSCs and improved the antitumor activity of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells in murine models [40]. Le et al. also reported that GEM decreased MDSCs in a murine mammary carcinoma model [41]. In addition, both 5-fluorouracil (5-FU) and docetaxel were reported to decrease splenic and intratumoral MDSCs without impairing immune cells [42, 43]. We also found that low-dose GEM decreased the number of MDSCs in tumor sites in murine colon carcinoma-bearing mice [44]. In contrast, some chemotherapeutic drugs were reported to promote tumor growth. GEM and 5-FU were reported to induce cathepsin B release-dependent inflammasomes in MDSCs and increase IL-1β production, which curtails antitumor immunity [45]. These results suggest an inconclusive effect of GEM and 5-FU on antitumor immunity.

3.4.3 DCs

As described above, anthracycline induces "immunogenic" tumor cell death and triggers DC maturation [10, 11]. Some chemotherapeutic drugs also have the potential to activate DCs. Tanaka et al. reported that chemotherapeutic drugs, including vinblastine, paclitaxel, and etoposide, promote DC maturation at nontoxic concentrations [46]. The same group further showed that the local injection of vinblastine at a low dose triggered the maturation of tumor-infiltrating DCs and thus stimulated antitumor immune responses in vivo [47].

3.4.4 Others

CTX has multifaceted effects on immunity. In addition to its effect on Treg cells, CTX influences DC homeostasis, type I IFN secretion, and the polarization of $CD4^+$ T cells into Th1 and/or Th17 cells [29]. Taxanes, including docetaxel, were reported to enhance cell-mediated antitumor activity and CTL function when combined with cancer vaccines [48, 49]. Moreover, several reports revealed that chemotherapy potentially renders cancer cells more susceptible to killing by CTLs. 5-FU, CPT-11, and cisplatin were shown to increase the sensitivity of human colon cancer cells to killing by T cells [50]. In addition, Ramakrishnan et al. showed that, in combination with a DC vaccine, paclitaxel increased tumor cell sensitivity to CTLs via dysregulation of cation-independent mannose 6-phosphate receptor, which is a receptor for granzyme B from CTLs, and endonuclease G, a protein that causes

caspase-independent DNA degradation [51]. Furthermore, Matar et al. reported that low-dose CTX decreased IL-10 and thereby altered the Th1/Th2 balance in favor of Th1 dominance [52, 53].

3.5 Effects of Metronomic Chemotherapy on Immunity

Although many studies have examined the effects of single injections of low-dose chemotherapeutic agents on immunity, there are few reports examining their effects when these agents are administered on a metronomic schedule. Ghiringhelli et al. reported that metronomic CTX reduced the frequency of circulating Treg cells as well as their immunosuppressive function and restored NK cell activity and T cell proliferation [5]. Importantly, this effect was observed only with low-dose CTX. Higher doses resulted in the depletion of all lymphocyte subpopulations. Generali et al. showed that letrozole combined with metronomic CTX significantly reduced the number of Treg cells in elderly breast cancer patients [54]. It was reported that metronomic low-dose CTX transiently reduced Treg cells but induced stable tumor-specific T cell responses in metastasized breast cancer patients [7]. In addition, Lord et al. performed a phase II study of low-dose metronomic oral CTX for hormone-resistant prostate cancer and reported its safety and effectiveness [6]. Alternatively, in a rat model of glioma, low-dose metronomic temozolomide resulted in the depletion and inhibition of Treg cell immunosuppressive activity [55].

Figure 3.2 summarizes the mechanisms by which metronomic chemotherapy suppresses tumor growth. In addition to suppressive effects on tumor angiogenesis,

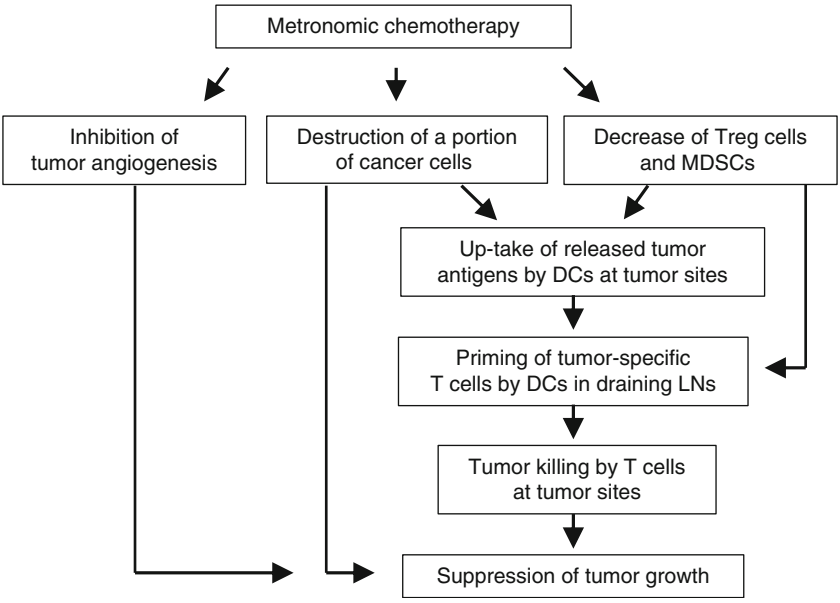

Fig. 3.2 Mechanisms of antitumor effects after metronomic chemotherapy

metronomic chemotherapy can destroy a portion of cancer cells, and tumor-infiltrating DCs can uptake tumor antigens from dying tumor cells. "Immunogenic" tumor cell death, explained above, could be induced even at low doses of chemotherapeutic agents. Simultaneously, drug-induced ablation of Treg cells and MDSCs can promote the antigen-presenting ability of DCs. After these DCs migrate to draining LNs and prime tumor-specific T cells, the primed and activated T cells traffic to tumor sites where they cause tumor cell lysis. Two different effects, the inhibition of tumor angiogenesis and immune cell-mediated cytolysis of tumor cells, synergistically exert antitumor effects.

3.6 Metronomic/Intermittent Chemotherapy with Low-Dose CTX and GEM

We recently reported that combination chemotherapy with low-dose CTX and GEM at 8-day intervals induced antitumor T cells in vivo through the mitigation of Treg cells and MDSCs [44]. The administration schedule of the 8-day interval cannot be termed metronomic because metronomic protocols refer to the administration of chemotherapeutic agents without prolonged drug-free periods. However, because our protocol relied on the repeated administration of chemotherapeutic drugs at low doses, we refer to it as "metronomic/intermittent chemotherapy" in this chapter.

As described above, CTX can decrease Treg cells in tumor-bearing hosts. On the other hand, CTX has the potential to kill proliferating lymphocytes. This implies that CTX can kill tumor-specific T cells after chemotherapy and suggests that CTX administration schedules should be carefully planned. We recently determined whether different administration protocols for low-dose CTX affect tumor-specific T cells [44]. We injected low doses of CTX into CT26 colon carcinoma-bearing mice and tested the antitumor T cell reactivity of the tumor-draining LN cells (Fig. 3.3). Although no reactivity was observed in the draining LN cells of naïve mice (group 1) or mice that were inoculated with CT26 14 days before (group 2), a tumor antigenic peptide-specific T cell response, which was evaluated by IFN-γ production, was observed in the draining LN cells from mice that were inoculated with CT26 14 days before and injected intraperitoneally with 100 mg/kg CTX 4 days before harvesting the LN cells (group 3). The tumor antigenic peptide-specific T cell response disappeared when the draining LNs were harvested 8 days after CTX injection (group 4). These results suggest that antitumor T cells were elicited on day 4 after CTX injection, but this T cell response disappeared thereafter. Importantly, no antitumor T cell response was observed in draining LN cells from mice that were inoculated with CT26 18 days before and injected intraperitoneally with half the CTX dose (50 mg/kg) twice [8 and 4 days before harvesting LN cells (group 5)]. Because CTX has the potential to kill proliferating T cells, the second injection of CTX, given 4 days after the first CTX injection, might destroy tumor-specific and proliferating T cells that were triggered by "immunogenic" tumor cell death after the first injection of low-dose CTX. Thus, these results imply that the administration schedule is critical for the subsequent induction of antitumor T cells in vivo.

To further enhance the antitumor effect of low-dose CTX, we combined low-dose CTX with low-dose GEM because these two agents can decrease tumor immunosuppressive cells (Treg cells and MDSCs, respectively). On day 10 after tumor

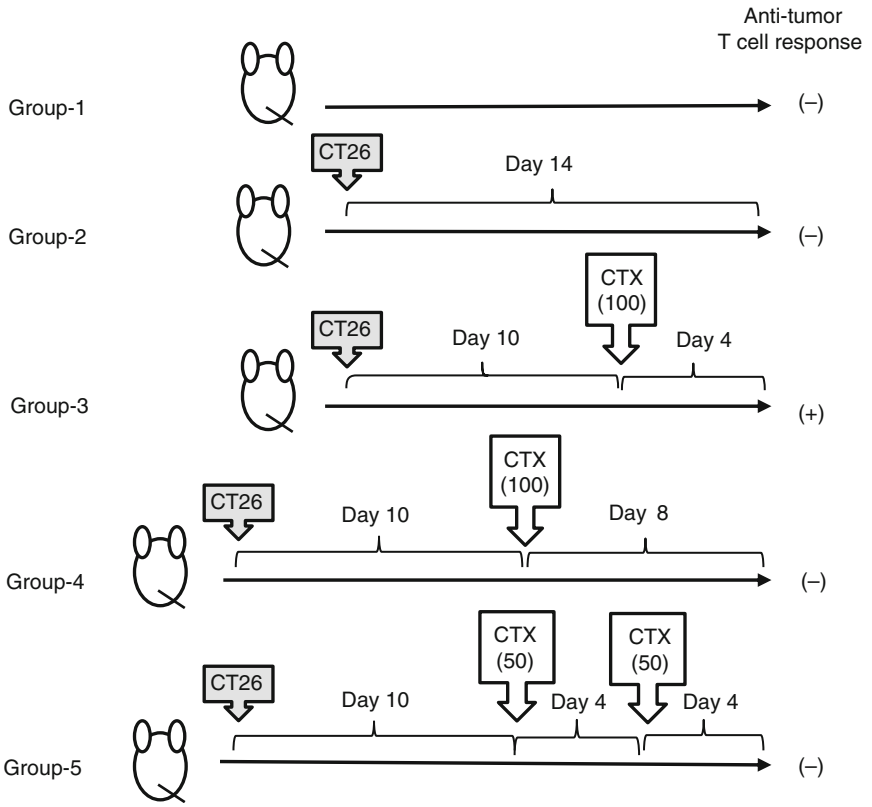

Fig. 3.3 Influences of different low-dose CTX protocols on the induction of antitumor T cells in vivo

inoculation, colon carcinoma-bearing mice were treated with metronomic/intermittent chemotherapy using low-dose 50 mg/kg CTX plus 50 mg/kg GEM at 8-day intervals. We found that tumor growth was significantly suppressed [44]. We confirmed that one injection of CTX or GEM at a dose of 50 mg/kg decreased the mRNA expression of Foxp3 and arginase-1, which are known markers of Treg cells and MDSCs, respectively, in tumor tissues. Co-injection of both drugs decreased Foxp3 and arginase-1 mRNA expression. In T cell-deficient nude mice, the antitumor effect induced by metronomic/intermittent chemotherapy with low-dose CTX and GEM was attenuated, implying that these effects are T cell dependent.

3.7 Influence of the Administration Schedule on Antitumor T Cell Responses

Because metronomic chemotherapy was primarily designed to target endothelial cells, chemotherapeutic drugs are administered without a drug-free period. However, as shown in Fig. 3.3, two injections of low-dose CTX at 4-day

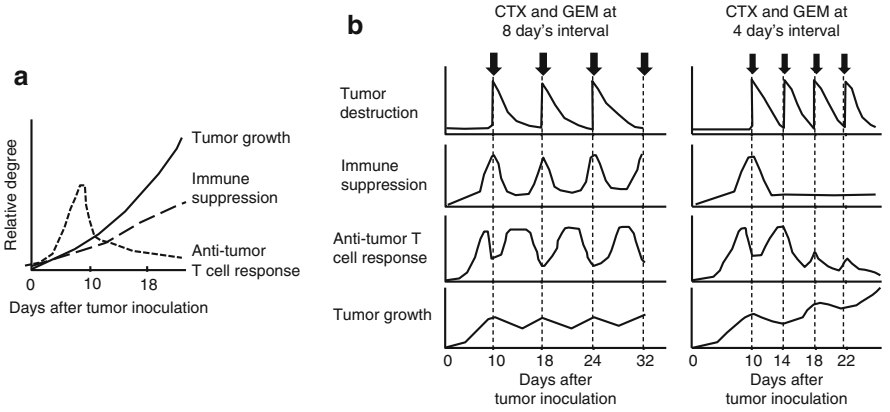

Fig. 3.4 Influences of different administration protocols of chemotherapeutic agents on the in vivo induction of antitumor T cells and tumor growth. (**a**) Kinetic changes in the antitumor T cell response, immune suppression, and tumor growth. (**b**) Influences of different administration protocols of chemotherapeutic agents on immunosuppression, the antitumor T cell response, and tumor growth

intervals ablated antitumor T cell immunity. These results indicate that the administration schedule of chemotherapeutic agents could significantly influence T cell immunity in tumor-bearing hosts. Moreover, the induction of tumor-specific T cells after chemotherapy requires a certain period of time. As shown in Fig. 3.4a, in a preclinical murine model, the antitumor T cell response in draining LNs was transiently induced approximately 1 week after tumor inoculation. However, Treg cells and MDSCs began to increase thereafter, thus inhibiting the antitumor T cell response and allowing the tumor to grow. One injection of low-dose CTX and GEM decreased the numbers of Treg cells and MDSCs, whereas two injections of these agents at 4-day intervals at half-doses inhibited the induction of antitumor T cells in vivo. In this regard, I propose a putative mechanism to explain why repeated injections of the chemotherapeutic agents at 4-day intervals failed to induce antitumor T cell immunity (Fig. 3.4b). When low-dose CTX and GEM are repeatedly administered at 8-day intervals, the chemotherapeutic agents destroy cancer cells and trigger "immunogenic" tumor cell death, leading to the in vivo induction of antitumor T cells. However, Treg cells and MDSCs increase again thereafter, and the next injection of the chemotherapeutic agents can inhibit the reemergence of Treg cells and MDSCs. As a result, tumor growth is continuously suppressed, leading to a stable state. In contrast, when low-dose CTX and GEM are administered at 4-day intervals, the situation is quite different. The first administration of agents can destroy cancer cells and trigger "immunogenic" tumor cell death. However, the next injection of agents, 4 days after the first injection, destroys or depletes tumor antigen-stimulated and proliferating T cells in vivo. Indeed, antigen-stimulated and proliferating T cells can be preferentially destroyed by subsequent injections of CTX [56]. Consequently, impaired T cell immunity in tumor-bearing hosts cannot control tumor growth.

Conclusions

I have introduced accumulating evidence that commonly used chemotherapeutic agents have the potential to restore and stimulate antitumor immune responses. Certain chemotherapeutic agents can decrease or ablate immunosuppressive cells, including Treg cells and MDSCs, and induce "immunogenic" tumor cell death. Because metronomic chemotherapy was primarily designed to target endothelial cells, low-dose chemotherapeutic agents are repeatedly administered without a drug-free period. I introduced data from our recent study showing that administration intervals significantly influence induction of the antitumor T cell response in vivo. Although some preclinical studies of metronomic chemotherapy have reported long-term tumor responses [57, 58], most of these immunodeficient mice eventually relapsed [59]. The use of immunologically incompetent mice might make it difficult to estimate the roles of host immunity in tumor-bearing hosts treated with metronomic chemotherapy. In my opinion, although the metronomic schedule is optimal for targeting circulating endothelial cells, there is a risk of inhibiting the induction of antitumor T cells after chemotherapy-induced "immunogenic" tumor cell death. Chemotherapeutic agents, even at low doses, can trigger tumor cell death and subsequent activation of DCs and tumor-specific T cells, and these processes require certain time intervals. In general, longer intervals might be needed in a clinical setting. For the induction of antitumor immunity in vivo after chemotherapy, administration schedules must be carefully designed with consideration of the interval required for the induction of antitumor T cells after chemotherapy-induced "immunogenic" tumor cell death.

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Preclinical Activity of Metronomic Regimens with Alkylating Agents and Antimetabolites

4

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Abstract

We know that the same drug, when administered at different doses, schedules, and moments, can produce completely different effects on tumor progression. For the last 10 years, research has been trying to unravel how metronomic chemotherapy antitumor effects arise.

Numerous in vitro and in vivo studies have provided evidence that the main effects of metronomic chemotherapy are related not only to tumor angiogenesis but also to the cancer cells, tumor environment, and stromal component. Nevertheless, there remain large gaps in our knowledge of the molecular mechanisms by which these effects arise.

This review summarizes part of the preclinical research, performed with those alkylating agents and antimetabolites most commonly used in the metronomic chemotherapy approach. Much of this report concerns cyclophosphamide, since, in this context, it is the most widely explored drug so far. The report also draws attention to the numerous cancer cell lines and the main murine models used.

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4.1 Alkylating Agents

Alkylating agents were one of the earliest classes of drugs used to treat cancer, emerging in the 1940s. The biggest weakness of most cancer cells is that they are very sensitive to DNA damage. Alkylating agents impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules. The most important sites for alkylation are DNA, RNA, and proteins. Alkylating agents depend on cell proliferation for activity but are not specific to the cell cycle phase. A fixed percentage of cells are killed at a given dose. Depending on their chemical structure and mechanism of covalent bonding, these drugs can be classified as nitrogen mustards (ifosfamide, melphalan, cyclophosphamide, chlorambucil), nitrosoureas (lomustine (CCNU), carmustine (BCNU), streptozocin), alkyl sulfonates (busulfan), triazines (dacarbazine, temozolomide), and ethylenimines (thiotepa), among other agents.

4.1.1 Cyclophosphamide

Cyclophosphamide (*CTX*) is a nitrogen mustard alkylating agent and a member of the oxazophorine group. It is the basis of many combination regimens for the treatment of lymphomas, leukemias, multiple myeloma, mycosis fungoides, neuroblastoma, retinoblastoma, and cancers of the breast and ovary. The wide variety of cancers it treats means that there is also a wide range of administering options. The most common methods are by intravenous injection or mouth in the form of tablets. It is usually administered intravenously at a dose of 600–750 mg/m^2/3 weeks. Its main toxicities are hematotoxicities and urinary toxicity.

CTX is a prodrug that requires the liver hepatocyte p-450 system to generate active cytotoxic metabolites, except 4-HC, which spontaneously converts to 4-hydroxy-CTX in aqueous solution and can then be used to perform in vitro studies [1]. Thus, the exposure of various cellular lines to 4-hydroxy-CTX suggests that this drug acts in different ways depending on the cell type. Furthermore, while the highest doses of the drug affect cancer cells and established fibroblasts, low doses of 4-hydroxy-CTX affect endothelial cells proliferation, inducing apoptosis and enhancement of the endogenous antiangiogenic factor trombospondin-1 (TSP-1) expression [2]. Acrolein is another derivate of CTX that alters the cytoskeleton of tumor cells to induce apoptosis (through NF-κ_B signaling) and upregulation of TSP-1 [3, 4].

Since the first metronomic in vivo studies, CTX has been the most widely studied agent because of its orally administered prodrug form. In mice, the CTX metronomic schedule is based on daily orally administration of 10–25 mg/kg (in drinking water or by gavage) without treatment breaks. In contrast, the maximum tolerated dose (MTD) is usually administered intraperitoneally at a dose of 100–150 mg/kg three times, once every other day or at 2-day intervals followed by 2 weeks of rest [5, 6].

Thus, in 2000, Bowder et al. demonstrated in Lewis lung carcinoma and L1210 leukemia that when administered at doses lower than the MTD, with shorter intervals and without extended rest periods, CTX could exert an angiogenic effect, resulting in tumor control for an extended period [7]. Similar findings were obtained with cyclophosphamide-resistant tumors (Lewis lung carcinoma and mammary carcinoma cell line

EMT-6), in which metronomically administered cyclophosphamide restored tumor sensitivity [7]. Klement et al. subsequently reported on the effect of low-dose continuous CT as a possible anti-angiogenic strategy in a mouse model of neuroblastoma [8]. At that point, Hanahan et al. coined the term "metronomic chemotherapy" (MC) to describe the concept of anti-angiogenic low-dose chemotherapy [9]. Metronomic chemotherapy is thought to exert its anticancer activity mainly by inhibiting tumor angiogenesis [10, 11]. In consequence, by targeting tumor endothelial cells, metronomic chemotherapy should indirectly destroy cancer cells by inducing hypoxia and starvation of nutrients.

Subsequent studies in tumor-bearing mice have confirmed the effect and the antiangiogenic basis of metronomic CTX in a variety of tumor types, including multiple myeloma [12], lymphoma [13, 14], melanoma [14], colorectal [9, 14, 15], pancreatic [14], prostate [15, 16], and breast [15] cancers.

The angiogenic effects described include microvessel density decrease and hypoxia induction [12, 17], inhibition of the ability of endothelial cells to form tubes [18], and induction of dividing endothelial cell apoptosis [1]. In addition, experimental studies have established that TSP-1 plays an important role in these effects. Thus, metronomic CTX has antitumor effects with high levels of TSP-1 in tumor-bearing mice, while the same treatment cannot induce this activity in TSP-null mice [1, 15, 19].

The growth of some tumors may depend on vasculogenesis. While tumor angiogenesis vessels are formed from the preexisting vasculature, vasculogenesis is the formation of new vessels from circulating blood endothelial progenitor cells (CEPs). CEPs are almost completely absent from healthy adults (except in women during the menstrual phase associated with the vascular remodeling process) but are detected in blood of patients with vascular disorders, inflammation, and cancer [20]. Actually, CEPs and other circulating blood endothelial cells (CECs) are valid quantitative surrogate markers of angiogenesis and antiangiogenic drug activity [21]. In 2003, Bertolini et al. showed how the MTD of CTX was associated with extensive CEP mobilization and drug resistance, while metronomic cyclophosphamide was correlated with a decrease in CEP and longer treatment response. Thus, they suggested that metronomic chemotherapy was a promising strategy for reducing vasculogenesis-dependent mechanisms of tumor growth [22]. Soon after, an inverse correlation of CTX dose administration (among other drugs) with this CEP decrease was found, suggesting that CEPs are pharmacodynamic biomarkers that can be used to determine the optimal biological dose of metronomic chemotherapy regimens [23]. Similarly, subsequent studies proposed the introduction of CTX metronomic schedules as an alternative to antiangiogenic agents in established combination treatments. Daenen et al. suggested the use of vascular disrupting agents (VDAs) with metronomic CTX instead of bevacizumab, especially in those patients who present inherent or acquired antiangiogenic drug resistance [24].

Cyclophosphamide is involved in many purported immunomodulatory mechanisms among which, it has been corroborated, is its role inhibiting the population of regulatory T cells (T_{REG}), which are also known as "suppressor T cells"; T_{REG} are $CD4^+CD25^+$ lymphocytes, enriched in FoxP3, glucocorticoid-induced TNF receptor, and cytotoxic T-lymphocyte-associated antigen-4 that can inhibit antigen-specific immune response. T_{REG} can thus inhibit the tumor immune response by suppressing the activity of both tumor-specific ($CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T helper cells) and tumor-unspecific effector cells (natural killer [NK] and NK T cells)

[25]. Using the T_{REG} multiple subsets described, several studies have associated the presence of $CD4^+CD25^+FoxP3^+$ T_{REG} in tumors with poor prognosis [26–28].

Impairment of T_{REG} activity by specific blockade or depletion can enhance the immune response to tumor-associated antigens [25]. In 1988, Berd et al. described that when cyclophosphamide was administered before vaccination, the vaccine effect was enhanced [29]. It was subsequently observed that the dose and sequence of drug administration in relation to vaccine delivery have an important influence on this effect [30, 31]. Several other studies have shown that low-dose cyclophosphamide can increase the tumor immune response by decreasing numbers and inhibiting the suppressive functions of T_{REG} cells and by increasing lymphocyte proliferation [32]. Thus, Ghiringhelli et al. showed that a single low dose of CTX in rats depleted $CD4^+CD25^+$ T cells and delayed the growth of colon carcinomas [33]. Other studies show that while the CTX MTD decreases the abundance of all T cell subsets, metronomic CTX efficiently inhibits tumor growth specifically through $CD4^+CD25^+$ T cell depletion [31]. Other studies reported that low doses of cyclophosphamide also reduced $FoxP3^+$T cell functionality [34].

Other immunomodulatory roles for metronomic CTX have been described, among which is a reduction of some immunosuppressive cytokines, such as TGF-β, IL-10, and IL-2 [35–37]; stimulation of IFN-gamma-producing natural killer T cell recovery [37]; dendritic cell maturation [37, 38]; memory T cell survival; and stimulation of galactin-1 expression in primary tumor, metastasis, and spleen cells [14].

It has been recently reported that metronomic chemotherapy has a role in blocking the metastatic process. In 2011, Jang et al. demonstrated that extended metronomic CTX significantly suppressed spontaneous pulmonary metastasis from hepatocellular carcinoma [12]. They showed how metronomic CTX suppresses the expression of MMP-2 and MMP-9 activity. Of all the metalloproteinases studied, these have been shown to be clearly associated with invasion and metastasis [39, 40]. Furthermore, they showed a significant reduction in the expression of MMP-14 and TIMP-2, which are both activators of inactive MMP zymogens [41].

The tumor microenvironment supports the niche necessary for the cancer stem cell (CSC) population to maintain its stem cell properties and functions [42]. The CSC hypothesis suggests that neoplastic clones are maintained exclusively by a small subpopulation of cells that give rise to phenotypically diverse cancer cells [43]. A small population of CSCs is potentially very important because it may be responsible for recurrence after cancer treatments, even when most of the cancer cells appear to be killed. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues and for the ultimate design of stem cell therapeutics. Considering this possibility, in 2007 Folkins et al. investigated whether different antiangiogenic therapies (including metronomic CTX) can reduce the brain tumor stem-like cell (TSLC) fraction of glioma tumors [44]. This study was the first to show that endothelial cells did indeed secrete factors that enhance the capacity of glioma cells to form tumor spheres in vitro and, secondly, that metronomic CTX causes a reduction in tumor TSLC fraction. They proposed that an antiangiogenic effect was responsible for this. Interestingly, neither MTD cyclophosphamide nor a strict antiangiogenic treatment (based on the mouse VEGFR2 targeting antibody DC101) alone was sufficient to reduce the TSLC fraction in the tumor, suggesting that an additional mechanism contributed to the metronomic CTX reduction of the TSLC population.

Consistent with these results, it was reported shortly afterwards that the cancer stem cell population increased with increasing CTX doses [45]. This study was done in mice inoculated with a hepatocellular carcinoma cell line. Furthermore, this effect was enhanced after several treated mouse generations, leading to cells with more self-renewal potential, proliferative activity, and clonogenicity in vitro.

Later, in another hepatocellular carcinoma murine model, it was confirmed that some tumor cells remained in mouse livers after metronomic CTX treatment [46]. These cells presented a semi-quiescent CSC phenotype ($CD13^+$) and were simultaneously able to produce tumors. The authors proposed a combination of metronomic CTX with a CD13 inhibitor to eradicate the residual disease [47]. In contrast, it was recently reported that long-term CTX selected for a cell fraction named "side population" (SP) [48]. These cells, identified by their ability to efflux Hoechst dye, proved to be resistant to chemotherapy, resembling CSCs and therefore another source of systemic disease relapse [49].

Otherwise, metronomic chemotherapy has already been suggested as a maintenance administration strategy that could be given after the MTD-based CT in a multitargeted schedule called "chemo-switch" (C-S). In 2004, Kerbel and Kamen highlighted the success of a low-dose maintenance therapy that is given after the standard schedule to treat children with certain types of cancer, suggesting that these two types of schedules were not mutually exclusive and could be considered for use in adults [50]. The ideas of combining metronomic chemotherapy with MTD-based chemotherapy or with agents targeting vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptors were brought together in a preclinical study by Pietras and Hanahan in the RIP-Tag2 mouse model of pancreatic islet cell tumorigenesis [51]. Similarly, Bell-McGuinn et al. showed a synergistic effect of cyclophosphamide administration following the C-S schedule in the same model [52]. Soon after, the superior efficacy of this dosing combination was described in murine models of prostate and breast cancers. This study also revealed how toxicity was not any higher than with the standard MTD [15]. Similar results were recently found in a pancreatic cancer model [6].

Finally, many studies have shown an enhanced effect of metronomic CTX when combined with other cytotoxic agents (doxorubicin, liposomal doxorubicin, paclitaxel, cisplatin, UFT), immunomodulatory agents, and molecular-targeted agents [18, 52].

4.1.2 Temozolomide (TMZ)

Temozolomide (TMZ) is a second-generation alkylating agent. It is a member of the imidazotetrazine class of drugs and has excellent oral bioavailability and good penetration across the blood–brain barrier. TMZ is associated with generally mild, noncumulative myelosuppression and is well tolerated in adults and children with cancer [53, 54]. It has demonstrated efficacy in the treatment of a variety of solid tumors, including primary malignant brain tumors and metastatic melanoma [55]. TMZ was initially licensed for the treatment of recurrent high-grade gliomas at a dose of 150–200 mg/m^2/day for 5 days every 28 days (5-day regimen) [56], but lower daily metronomic doses (75 mg/m^2) are now also used. It remains highly controversial whether an optimal TMZ regimen actually exists [55].

The first preclinical studies of metronomic TMZ were done in 2003, after TMZ metronomic schedules had proved their antitumor efficacy in phase I trials [57]. In this preliminary work, an antiangiogenic effect of low doses of TMZ was confirmed in human umbilical vein endothelial cells (HUVECs). These results have recently been confirmed in vitro by Ko et al., who also showed a reduction of cell migration and angiogenic tube formation in HUVECs treated with metronomic TMZ [58]. Furthermore, the authors found that this schedule downregulated O^6-methylguanine-DNA-methyltransferase (MGMT), which is a DNA repair protein with a pivotal role in cellular resistance to alkylating agents [59]. These results have been confirmed in a TMZ-resistant cell line [60].

In 2006, in vivo studies showed that metronomic TMZ inhibited angiogenesis and augmented tumor cell apoptosis in a TMZ-resistant C6/LacZ rat glioma model, suggesting that this schedule can overcome the chemoresistance usually found after conventional TMZ chemotherapy in the clinic [61]. Later studies proved that lowering the TMZ dose did not alter the pharmacokinetic and pharmacodynamic parameters, providing a basis for further investigation of these regimens [62]. As for CTX, other effects of metronomic TMZ schedules began to be explored. Thus, the study of various TMZ regimens in Treg cell populations in a TMZ-resistant rat model of glioma again showed Treg depletion induced by the low-dose metronomic TMZ regimen, but not by the standard treatment. This effect was accompanied by a decreased suppressive function of the remaining Treg cells [63].

The endothelial and cancer effects of metronomic TMZ could be enhanced when combined with other antiangiogenic strategies such as sorafenib [64]. The overall findings therefore highlight the merits of metronomic dosing of TMZ in the clinical setting [65–67].

4.2 Antimetabolites

Antimetabolite drugs were among the first effective chemotherapeutic agents discovered. They are folic acid, pyrimidine, or purine analogues, characterized by low molecular weights and have structures similar to those of naturally occurring molecules used in nucleic acid (DNA and RNA) synthesis. They exert their cytotoxic activity by competing with normal metabolites for the catalytic or regulatory site of a key enzyme or by substituting for a metabolite that is normally incorporated into DNA and RNA. This mechanism of action means that antimetabolites are most active when cells are in the S phase and have little effect on cells in the G_o phase. Consequently, these drugs are most effective against tumors with a high growth fraction. Antimetabolites have a nonlinear dose–response curve, such that beyond a certain dose, no more cells are killed (5-FU being an exception). These agents are used for a variety of cancer therapies, including leukemia and breast, ovarian, and gastrointestinal cancers.

4.2.1 Gemcitabine (Gemcitabine HCl)

Gemcitabine (gemcitabine HCl) is the hydrochloride salt of the pyrimidine deoxycytidine. It is used to treat pancreatic cancer that is advanced or has spread, but in combination with other drugs, it is also used to treat advanced or spread breast,

ovarian, and non-small cell lung cancers. It is usually administered at a standard dose of 1,000–1,250 mg/m^2 by a 30-min intravenous infusion once a week for 3 or 4 weeks and has common hematological side effects.

Initial preclinical studies on metronomic gemcitabine focused on reducing the adverse clotting events usually found after chemotherapy treatment alone or in conjunction with antiangiogenic drug combination therapies [68]. The authors showed that reducing the concentration of gemcitabine in vitro significantly attenuated the increase in the coagulation index.

Most in vivo studies have employed orthotopic pancreatic cancer models. Generally, for one month of treatment, the MTD used has been around 100–120 mg/kg on 4 occasions at 3-day intervals [69–71], and the metronomic dose has been around 1 mg/kg daily [71, 72].

In 2008, Laquente et al. demonstrated the antiangiogenic effect of metronomic gemcitabine in an orthotopic pancreatic cancer model [71]. While the antitumoral effect was equivalent to that of the standard administration, metronomic gemcitabine produced a reduction in microvessel density that was correlated with an induction of thrombospondin-1. At the same time, the authors determined the gemcitabine metronomic schedule in mice as 1 mg/kg/day for 1 month (as a single treatment cycle). These dosing schedules were determined by in vitro and in vivo experiments in immunocompetent mice. In another study, also done in pancreatic cancer xenografts, metronomic gemcitabine produced a marked reduction in tumor levels of various proangiogenic molecules (including EGF, IL-1α, IL-8, ICAM-1, and VCAM-1), decreased tumor hypoxia, improved tissue perfusion, and increased cancer-associated fibroblast apoptosis [73]. In this case, for one cycle of treatment, dosing schedules were 240 mg/kg three times at weekly intervals for the gemcitabine MTD and 30 mg/kg every 3 days [73]. The variation of dosing schedules hinders the interpretation and comparison of the results. In this context, it is important to determine the correct metronomic dose for each drug.

Metronomic gemcitabine is known to inhibit multisite tumor metastasis [72]. The study shows an improvement of this effect when combined with sunitinib [72]. Soon after, similar results were obtained by Vives et al. [6], who reported an antiangiogenic effect and a significant inhibition of the cancer stem cell population underlying this effect.

Similar to cyclophosphamide, the combination of the two gemcitabine regimens integrated in the C-S schedule was also examined in this work. Not only a synergistic antitumoral effect (almost twice the degree of tumor growth inhibition as with MET or MTD administration) but also a blocking effect on tumor dissemination and a decrease of the cancer stem cell population caused by metronomic treatment was confirmed for the C-S schedule. These effects were achieved with no increased toxicity [6].

Immunomodulatory roles for metronomic gemcitabine have also been reported. While inhibition of TGF-β receptor I kinase or a CCR4 antagonist failed to abrogate Treg accumulation in pancreatic adenocarcinoma, metronomic gemcitabine selectively depleted Treg [74]. Similarly, it has been described that, combined with CTX, metronomic gemcitabine decreases the abundance of Tregs and myeloid-derived suppressor cells (MDSCs) [75]. These results provide a basis for new modalities in pancreatic cancer therapy.

Given such findings, the oral administration of gemcitabine should allow the drug to be given on a more frequent basis, reproducing these findings in the clinical environment. An oral prodrug is currently under clinical development [76, 77].

4.2.2 Fluorouracil (5-FU)

Fluorouracil (5-FU) is a pyrimidine base containing a fluoride atom at the 5-carbon position on the ring. It is used to treat several types of cancer, including those of the colon, rectum, and head and neck. It may also be used to treat skin cancers (basal cell and keratosis) by topical application. In 2004, Drevs et al. assessed the IC_{50} of 5-FU and other oral drugs in a murine renal cell carcinoma model [78] and found it to be more active against endothelial cells than tumor cells and to exhibit a G-1 arrest.

Even though orally administered 5-FU forms are known to have erratic absorption and nonlinear pharmacokinetics [79], investigators have a renewed interest in them in their development of continuous infusion treatments [80]. Similarly, the metronomic approach has been pursued through the use of various oral 5-FU prodrugs that have also shown some advantages in terms of antitumoral and antiangiogenic activity [81] or higher intratumor and plasma 5-FU concentration [82, 83]. To date, most of the findings described have involved the combination of 5-FU oral forms with other strategies.

4.2.3 Tegafur–Uracil (UFT)

Tegafur–Uracil (UFT) has been administered orally at very low doses as an adjuvant therapy, with favorable results [21]. In the preclinical environment, metronomic UFT delays the emergence of treatment resistance when combined with sunitinib in a hepatocellular carcinoma model [84]. UFT showed a synergistic antitumor effect when combined with CTX in an advanced metastatic breast cancer model [85]. In this study, the combination was superior to both monotherapies, and, interestingly, when the treatment was solely with UFT, there was no tumor invasion of the adjacent normal musculature, suggesting a possible malignancy effect of metronomic UFT. Consistent with the effectiveness of the combination of UFT and CTX, it was subsequently reported that CTX inhibited the dihydropyrimidine dehydrogenase (DPD) [86], which is the primary and rate-limiting enzyme involved in 5-FU metabolism [80]. Other combinations have yielded good results, for instance, with the aromatase inhibitor anastrozole (ANA) [87].

4.2.4 Capecitabine

Capecitabine is another orally administered prodrug of 5-FU that selectively delivers this agent to tissues, such as tumors, that express high levels of thymidine phosphorylase (TP). Metronomic capecitabine has proven antiangiogenic effects in tumor models of breast cancer [88] and of colorectal cancer with an induction of TSP-1 [89]. However, in these studies the administration of capecitabine was at a sub-MTD, which means a lower dose, but not a strictly metronomic one.

Several studies have confirmed TSP-1 induction by metronomic administration of other 5-FU prodrugs, such as *S-1* [89, 90], and propose their combination with

Table 4.1 Non-exhaustive list of preclinical research on metronomic chemotherapy using particular alkylating agents and metabolites

	Alkylating agents		Antimetabolites	
	CTX	TMZ	Gemcitabine	5-FU oral forms
Breast cancer	[2, 15, 23, 24]		[76]	[85, 87, 88, 92]
Lung cancer	[1, 7, 39, 40]		[77]	
Leukemia	[7]			
Neuroblastoma	[8]			
Multiple myeloma	[37, 93]			
Lymphoma	[13, 14, 20, 22, 35, 36]			
Melanoma	[1, 18, 23, 24, 39, 40]	[64]		
Colorectal cancer	[9, 14, 15, 17, 18]			[89, 91]
Pancreatic cancer	[6, 14, 18, 51, 52]		[6, 70–74]	
Prostate cancer	[15, 16, 38]			
Hepatocellular carcinoma	[12, 45, 46]			[84, 90]
Spontaneous erythroleukemia	[23]			
Hepatoma	[31]			
Colon cancer	[33, 75]		[75, 77]	
Glioma	[44]	[60–63]		
Esophageal cancer	[48]			
Ovarian cancer	[6]		[69, 76]	
Sarcoma			[69]	
Renal cell carcinoma				[78, 81]
Gastric cancer				[83]

other strategies, for example, the oral antiangiogenic drug vandetanib [90] or polyethylene glycol (PEG)-coated "neutral" liposomes, for which metronomic S-1 improved the intratumoral accumulation [91].

4.3 Concluding Remarks

Increasing amounts of information from preclinical research, much of which involves alkylating agents and antimetabolites, suggests that metronomic chemotherapy is a multitargeted and effective approach involving a wide variety of drugs and tumors with different biology and degrees of aggressiveness (Table 4.1). Nevertheless, there is still much to be explored in this field, for instance, the optimization of appropriate drugs, doses, schedules, and mechanisms of action. Given its lower toxicity profile, metronomic chemotherapy could be used as an additional treatment to conventional MTD chemotherapy, bringing new dosing schedules like the named chemo-switch (Fig. 4.1). Furthermore, it can be combined not only with antiangiogenic agents but also with a wide range of treatment strategies. Together with its low cost and the development of oral forms, metronomic chemotherapy represents an attractive cancer therapeutic approach.

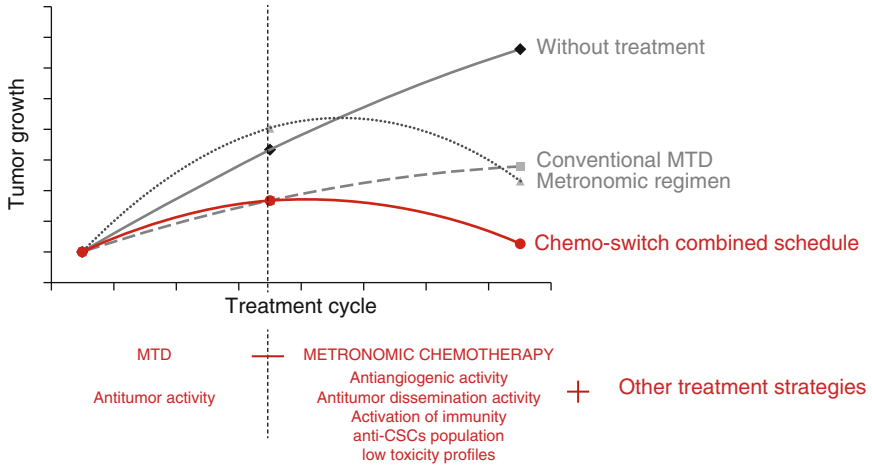

Fig. 4.1 Multitargeted metronomic chemotherapy given after the antitumor MTD in a combined chemo-switch schedule

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Metronomic Chemotherapy Regimens Using Microtubule-Targeting Agents: Mechanisms of Action, Preclinical Activity and Future Developments

5

Eddy Pasquier, Maria Kavallaris, and Nicolas Andre

Abstract

Microtubule-targeting agents (MTAs) are amongst the most successful chemotherapeutic drugs commonly used in the clinic for the treatment of human cancers. Although originally administered at or close to the maximum tolerated dose once every 3 weeks, the discovery of their potent antiangiogenic properties at the end of the 1990s has led to the re-evaluation of treatment protocols. Nowadays, MTAs are often administered at lower doses either weekly or even more frequently following a metronomic schedule, thus leading to increased efficacy and decreased toxicity. In this chapter, we present an overview of the in vitro and

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in vivo studies that have contributed to the development of MTA-based metronomic chemotherapy protocols and increased our understanding of their mechanisms of action. First, we discuss the complex cellular and molecular mechanisms involved in the antiangiogenic activity of MTAs. We also present their effects on the immune system, which may contribute to the antitumour efficacy of MTA-based metronomic chemotherapy. Then, we review the results obtained with this type of therapeutic approach in preclinical models of human cancer, focusing on the most promising combination treatments. Finally, we oversee the future developments in this field in terms of new MTAs and novel formulations currently in development with the aims to improve efficacy and bioavailability while increasing tumour targeting and specificity.

5.1 Introduction

Microtubule-targeting agents (MTAs) are amongst the most successful classes of chemotherapeutic drugs currently used in the clinic for cancer treatment. They are part of the standard of care for a wide variety of human malignancies, including breast, ovarian, prostate, head and neck, lung and endometrial cancers as well as leukaemia [1–4]. They are usually divided into two distinct categories based on their effects on the microtubule cytoskeleton: microtubule-stabilising agents and microtubule-depolymerising agents. The main stabilising compounds used in the clinic are the taxanes, paclitaxel and docetaxel, while the main depolymerising agents belong to the *Vinca* alkaloid family, such as vincristine, vinblastine, vinorelbine and the newest derivative vinflunine. In addition, numerous MTAs are in various stages of preclinical and clinical development, including microtubule-stabilising agents epothilones and microtubule-depolymerising agents combretastatins. Since their original introduction in the cancer armamentarium 50 years ago, MTAs have mostly been used in the clinic following conventional administration schedules: once every 3 weeks, at or close to the maximum tolerated dose (MTD). However, the discovery of their potent antiangiogenic properties in the 1990s and the emergence of the concept of metronomic chemotherapy in 2000 have led to the re-evaluation of these administration schedules. Nowadays, these drugs are often administered weekly or even more frequently as part of metronomic combination protocols.

The effects of MTAs on the tumour vasculature have been reviewed extensively over the past 10 years [5–8]. In this chapter, we will summarise the cellular and molecular mechanisms involved in the antiangiogenic properties of MTAs and describe their effects on antitumour immunity. We will then review the activity of metronomic MTAs alone and in combination with other therapeutic strategies in preclinical models and finally foresee the future developments in terms of new agents and novel formulations that will contribute to the progression of this research field in the coming years.

5.2 Molecular Insights into the Anti-vascular Properties of MTAs

Major insights have been gained in recent years into the complex molecular and cellular mechanisms involved in the anti-vascular activity of MTAs. These effects can be classified in two categories: antiangiogenic effects (i.e. inhibition of novel blood vessel formation) and vascular-disrupting effects (i.e. rapid collapse of existing tumour blood vessels). Interestingly, virtually all MTAs display relatively potent antiangiogenic properties, whereas only microtubule-depolymerising agents possess vascular-disrupting properties [6, 7, 9]. The deciphering of the anti-vascular properties of MTAs has not only guided the clinical utilisation of these drugs and the optimisation of treatment schedules, but it has also revealed the key role played by the microtubule network in tumour angiogenesis.

5.2.1 Effects of MTAs on the Microtubule Network of Vascular Endothelial Cells

Microtubules are highly dynamic structures that permanently alternate between phases of polymerisation, pause and depolymerisation, and their dynamic properties are finely regulated [10]. These dynamic properties are critical for the different cellular functions of microtubules, ranging from cell division, cell shape maintenance, intracellular trafficking, extracellular secretion, cell signalling and motility. Angiogenesis is a complex multistep process that involves activation, migration, invasion, proliferation and morphological differentiation of endothelial cells into capillary tubes, all of which rely on a very dynamic and tightly regulated microtubule network [11]. It is therefore not surprising that MTAs hold such potent antiangiogenic properties.

Although angiogenesis inhibition can occur at cytotoxic concentrations of MTAs [12, 13], studies rapidly showed that these antiangiogenic effects are often maximal at very low non-cytotoxic concentrations [12–15]. MTAs can thus inhibit the migration of endothelial cells and formation of vascular structures in vitro at very low concentrations that affect neither their proliferation nor the structural organisation of their microtubule network [9, 16–19]. This is in sharp contrast with the vascular-disrupting activity of microtubule-depolymerising agents, which is often associated with extensive microtubule depolymerisation followed by rapid vascular collapse [6, 7, 20]. The lack of significant structural modifications of the microtubule network induced by low antiangiogenic concentrations of MTAs has prompted cancer biologists to seek alternative mechanisms.

The reorientation of the microtubule-organising centre (MTOC) in the intended direction of movement is an early and critical step in cell migration and motility [21, 22]. Using an in vitro wound healing experiment, Hotchkiss and colleagues reported that the inhibition of endothelial cell migration induced by docetaxel was

associated with an impairment of the reorientation of the MTOC [16]. However, no significant change in MTOC orientation was observed in a recent study investigating the impact of both paclitaxel and docetaxel on the migration of endothelial cells using a very similar experimental procedure [23]. This discrepancy suggests that the inhibition of MTOC reorientation may not play a critical role in the antiangiogenic activity of MTAs.

Although alternative mechanisms have been reported, MTAs are generally thought to exert their anticancer effects by suppressing microtubule dynamics in cancer cells, which prevents the formation of a functional mitotic spindle, resulting in cell cycle arrest at the metaphase to anaphase transition and subsequent apoptosis induction [1]. Ten years ago, we were the first to investigate the effects of MTAs on the microtubule dynamics of vascular endothelial cells in order to better understand their antiangiogenic properties. Since endothelial cells are extremely sensitive to MTAs [15, 17], we originally hypothesised that their microtubule dynamics would be suppressed at very low drug concentrations that would not have any effect on the microtubule dynamics of cancer cells and other cell types. Unexpectedly, however, we found that low antiangiogenic concentrations of MTAs induced a significant increase in the dynamic behaviour of microtubules in endothelial cells [19, 24]. The percentage of dynamic microtubules and their overall dynamicity were markedly increased at low non-cytotoxic concentrations and suppressed at higher cytotoxic concentrations in endothelial cells, whereas microtubule dynamics were either unaltered or decreased at all tested drug concentrations in cancer cells. We confirmed these results in two different endothelial cell lines, using two different MTAs (i.e. paclitaxel and vinflunine) and two distinct experimental procedures (i.e. microinjection of rhodamine-labelled tubulin and transfection with GFP-tubulin). More recently, Honore and colleagues found a significant correlation between the anti-migratory effects of vinflunine at low concentration in endothelial cells and the reduction in the length of comet-like structures formed by microtubule-binding protein EB1 at the microtubule (+) ends [25, 26]. This effect on EB1 localisation was associated with reduced detyrosination of EB1, further supporting an increase in microtubule dynamics induced by low antiangiogenic concentrations of MTAs in endothelial cells. In contrast, two recent studies investigating the regulation of microtubule dynamics in migrating endothelial cells reported a decrease in microtubule dynamics induced by MTAs (i.e. paclitaxel, vinblastine and colchicine) at concentrations that significantly inhibited endothelial cell migration [23, 27]. Although contradictory in appearance, this discrepancy in results may be explained by differences in experimental conditions. For instance, Ganguly et al. transfected endothelial cells with EGFP-MAP4 (a microtubule-associated protein) to analyse microtubule dynamics [27], whereas we and others have used either microinjection of rhodamine-labelled tubulin or transfection with GFP-tubulin, mCherry-tubulin and GFP-EB1 [19, 24–26]. Similarly, Kamath et al. analysed the dynamics of microtubules in confluent endothelial cells seeded on fibronectin-coated glass coverslip, serum starved for 24 h and migrating towards a cell-free wound [23], while other studies have used endothelial cells sparsely seeded onto uncoated glass coverslip, in 10 % foetal calf serum and undergoing random motility [19, 24–26].

In addition, it is interesting to note that although not statistically significant, these two more recent studies observed modest increases in microtubule dynamics at the lowest drug concentration tested [23, 27]. Perhaps more importantly than the slight discrepancies in results, all these in vitro studies have revealed that microtubule dynamics are extremely sensitive to experimental conditions and external stimuli and tightly regulated within cells. For instance, microtubules at the trailing edge of migrating endothelial cells were found to be twice as dynamics as those in the cell leading edge [27]. Furthermore, VEGF was shown to reduce microtubule dynamics by 50 % and decrease the length of EB1 comets by 40 % in endothelial cells, while treatment with a VEGF trap resulted in a significant increase in microtubule dynamics [26].

Overall, the multistep process of angiogenesis requires extreme plasticity of the endothelial cytoskeleton [11]. The microtubule network of endothelial cells is therefore tightly regulated by multiple internal factors and external stimuli, and the dynamic behaviour of microtubules can be either decreased or increased depending on the context. By interfering with this complex regulatory mechanism of microtubule dynamics, MTAs are able to hinder the migration and morphological differentiation of endothelial cells and induce highly potent antiangiogenic effects at very low concentrations.

5.2.2 Additional Mechanisms

Besides their effects on the microtubule network of endothelial cells, a number of additional mechanisms involved in the antiangiogenic activity of MTAs have been identified over the past 10 years. Some of these mechanisms are directly related to the impact of metronomic MTAs on microtubules, while other mechanisms appear to be independent or indirectly related.

Microtubules are known to regulate the turnover of adhesion sites [10]. As a result, the antiangiogenic and anti-migratory effects of low-dose MTAs have been associated with inhibition of trailing edge retraction in migrating endothelial cells [27] and accumulation of actin stress fibres and large and disorganised adhesion sites [9, 25]. Consistently, disturbance of the Rho GTPase signalling pathway through the inhibition of Rac1 and Cdc42 has also been reported in endothelial cells treated with low-dose MTAs [28].

Elsewhere, the microtubule cytoskeleton also plays a critical role in post-transcriptional regulation and translation through its involvement in mRNA and protein transport [29]. It is therefore not surprising that the antiangiogenic activity of MTAs has been associated with important changes in the expression of key factors involved in the regulation of tumour angiogenesis. For instance, Bonezzi et al. recently showed that the anti-migratory effect of paclitaxel in endothelial and cancer cells was associated with nuclear translocation of transcription factor FOXO3a and induction of tubulin acetylation, most likely through inhibition of tubulin deacetylase SIRT2 [30]. Similarly, both microtubule-depolymerising (i.e. vinblastine, 2ME2 and its analogue ENMD-1198) and microtubule-stabilising (i.e.

paclitaxel and epothilone B) agents have been reported to interfere with hypoxia signalling in endothelial and cancer cells, by reducing HIF-1α expression and/or nuclear accumulation [31–33]. Consistently, low-dose MTAs have been shown to inhibit the transcriptional activation of VEGF expression and reduce VEGF levels in vitro and in vivo [31, 33–38]. Furthermore, Murtagh et al. demonstrated that the anti-migratory effects of low-dose docetaxel were, at least in part, mediated by the ubiquitination and subsequent proteasomal degradation of HSP90 as a result of its dissociation from the microtubules [39]. In addition, Bocci et al. demonstrated that protracted low dose of chemotherapy agents, including paclitaxel, induced a profound increase in the expression of endogenous angiogenesis inhibitor thrombospondin 1 (THBS1) in endothelial cells in vitro [40]. This effect was further confirmed in rat bearing prostate tumours not expressing THBS1, where continuous treatment with low-dose paclitaxel resulted in re-induction of THBS1 [41]. Increased expression of THBS1 has also been reported since then in several preclinical studies investigating the efficacy of metronomic taxanes alone or in combination with other therapeutic strategies [37, 42, 43]. MTAs have also been shown to downregulate VEGFR-2 expression through transcriptional and post-transcriptional mechanisms. This effect was observed in vitro with both microtubule-stabilising (i.e. nocodazole) and microtubule-destabilising agents (i.e. vinblastine, 2ME2 and its analogue ENMD-1198) [9, 44]. Furthermore, Jiang et al. have also reported a decrease in VEGFR-2 expression in murine breast tumours in vivo following treatment with metronomic paclitaxel [43]. Thus, the role of SIRT2, HIF-1α, VEGF, HSP90, THBS1 and VEGFR-2 in the mechanism of action of metronomic MTAs has been established. This may open new avenues for the development of combination therapies using metronomic MTAs and, for instance, antiangiogenic compounds, sirtuin inhibitors or HSP90 inhibitors. However, these findings are yet to translate into the successful implementation of any of these factors in the clinic as reliable biomarkers for patient selection and/or treatment monitoring [45].

Additional mechanisms involved in the anti-vascular properties of MTAs include transient disturbances of mitochondrial metabolism, increased drug uptake, inhibition of endothelial progenitor cell (EPC) mobilisation as well as long-term chemosensitisation and sustained impairment of the angiogenic potential of endothelial cells. First, we found that low-dose paclitaxel induced a cytostatic effect in endothelial cells, which was associated with the initiation, without completion, of the mitochondrial apoptotic pathway [18]. In particular, incubation with low antiangiogenic concentrations of paclitaxel resulted in an early increase in the mitochondrial membrane potential, Bax/Bcl-2 ratio and p53 expression, indicating an activation of the mitochondrial apoptotic pathway. However, these effects were transient and did not translate into apoptosis induction and only slowed endothelial cell proliferation. The implication of mitochondria in the anti-vascular activity of paclitaxel is particularly interesting in light of the potent antiangiogenic properties of antimitochondrial drugs [46]. Elsewhere, Merchan et al. demonstrated that paclitaxel can accumulate up to five times more inside endothelial cells than in normal fibroblast and cancer cell lines [47]. Interestingly, we observed a similar increased intracellular uptake of vincristine inside bone marrow-derived endothelial cells as compared

to neuroblastoma cells [48]. Shaked et al. investigated the impact of MTAs on EPC mobilisation in various tumour models. They first showed that both metronomic vinblastine and vinorelbine were able to decrease the number of viable circulating EPC and that this effect was associated with optimal antiangiogenic and antitumour effects [49]. In contrast, they demonstrated that MTD paclitaxel induced a rapid mobilisation and subsequent tumour homing of EPCs, which most likely contribute to tumour regrowth during drug-free breaks [50]. Interestingly, Muta et al. recently found that metronomic paclitaxel and docetaxel were able to block EPC mobilisation and tumour homing [51]. Taken together, these studies clearly show that MTAs administered following a metronomic schedule, but not at the MTD, can block tumour vasculogenesis and regrowth induced by EPCs. Finally, we recently investigated the impact of long-term treatment with metronomic and MTD vinblastine on endothelial cells [48]. We found that while repeated exposure to MTD vinblastine induced some level of drug resistance in endothelial cells, metronomic vinblastine conversely increased their chemosensitivity and decreased their angiogenic potential. This effect was associated with decreased expression of βIII-tubulin in endothelial cells, an important factor involved in drug resistance in lung and ovarian cancer [52].

5.3 Beyond Angiogenesis Inhibition

We recently highlighted the fact that metronomic chemotherapy is more than just an antiangiogenic therapy [53]. In recent years, additional mechanisms have been unveiled, thus opening new avenues for the optimisation of treatment protocols as well as for the development of novel combinatorial strategies. One of the key mechanisms that have recently emerged is the positive impact of metronomic chemotherapy on antitumour immunity.

First, Machiels et al. showed that paclitaxel was able to enhance the antitumour efficacy of a whole-cell vaccine by amplifying the T-cell response in a breast cancer model [54]. Vicari et al. also demonstrated that paclitaxel increased the efficacy of a Toll-like receptor 9 (TLR9) agonist in an orthotopic model of renal cell carcinoma, by reducing the number and inhibiting the activity of regulatory T cells (Tregs) in a TLR4-independent manner [55]. This synergism was associated with a decrease in IL-10 expression and increase in IL-17-secreting CD4+ T cells. Recently, the impact of paclitaxel on Tregs was further confirmed in a mouse model of Lewis lung carcinoma [56]. Similarly, docetaxel was found to decrease the levels of myeloid-derived suppressor cells (MDSCs) in the spleen of breast tumour-bearing mice, which led to enhanced antitumour cytotoxic response [57]. In sharp contrast, docetaxel and paclitaxel were also shown to stimulate the secretion of monocyte chemoattractant protein 1 (MCP1 – also called CCL2) by tumour cells [58, 59], which facilitates the recruitment of macrophages and the establishment of an immunosuppressive stroma [60]. Elsewhere, paclitaxel can also increase the permeability of tumour cells to granzyme B, thereby rendering them susceptible to cell lysis induced by cytotoxic T cells (CTLs) even when the tumour cells do not express

the antigen recognised by the CTLs [61]. This bystander effect may play a crucial role in the efficacy of strategies combining metronomic MTAs with immunotherapy (see Sect. 5.4.2).

Microtubule-depolymerising agents have also been associated with important immunomodulatory effects. For instance, vincristine administered in combination with doxorubicin and glucocorticoids has been shown to increase the abundance of specific dendritic cell (DC) subsets in patients with multiple myeloma [62]. Interestingly, both vincristine and paclitaxel administered at low dose can stimulate antigen presentation by DC in an IL-12-dependent mechanism [63]. In addition, a study by John et al. showed that DCs displayed a significant level of resistance to paclitaxel and that this drug can increase the expression of MHC class II molecules on these cells and subsequently stimulate the proliferation of allogeneic T cells in vitro [64]. Furthermore, low doses of vinblastine and paclitaxel were found to stimulate the maturation of DC in vitro [65], and injection of vinblastine at low doses could trigger the maturation of tumour-infiltrating DC in vivo, thus stimulating antitumour immune response [66]. The effect of low-dose MTAs (i.e. vincristine, vinblastine and paclitaxel) on DC maturation was further confirmed by Shurin and colleagues [67]. Finally, Wan et al. recently showed that both paclitaxel and vinblastine induced elevated expression of MHC class I molecule and increased secretion of IFN-β by breast cancer cells [68].

Despite these recently uncovered immunostimulatory effects of MTAs, it is important to note that these drugs also exert numerous deleterious effects on the immune system. Besides the well-characterised neutropenia and leukopenia associated with MTAs administered following a conventional schedule (i.e. at or close to the MTD), vinorelbine, for instance, was also recently shown to cause bystander death of immune cells through the induction of cellular oxidative and nitrosative stress in lung carcinoma cells in vitro and in vivo [69].

Overall, MTAs induce a plethora of effects on the different actors of the immune system, as recently reviewed by Galluzzi et al. [70]. Most of these effects appear to be dose dependent, and immunostimulatory effects seem to be maximised with the use of low dose and repeated exposure [71], thus paving the way for the combination of metronomic MTAs with immunotherapy.

5.4 Preclinical Studies

Since the publication of the two seminal studies that led to the inception of the field of metronomic chemotherapy in 2000 ([72, 139]), a large number of in vivo studies have investigated the antitumour efficacy of MTAs administered following a metronomic schedule. Drugs that have shown activity in preclinical models when used metronomically include taxanes, *Vinca* alkaloids as well as newer MTAs, such as epothilones and 2-methoxyoestradiol (2ME2) [6]. Metronomic MTAs were found to induce potent antitumour and antiangiogenic effects in a variety of animal models of human malignancies, including neuroblastoma [72] and breast [43, 49, 51, 73], colorectal [74], prostate [41] and gastric cancer [37]. Recently, the antiangiogenic

properties of metronomic epothilone B were also demonstrated using an innovative ex vivo assay based on outgrowth of capillary tubes from fresh human tumour samples [75]. Although metronomic MTAs alone displayed promising activity in various preclinical models, they are most likely to be associated with other types of therapeutic strategies in the clinic [45]. Therefore, here we will focus on the most promising combinatorial approaches involving the use of MTAs administered metronomically.

5.4.1 Metronomic MTAs in Combination with Targeted Agents

One of the two initial publications on metronomic chemotherapy was based on continuous administration of vinblastine at low dose alone or in combination with an anti-VEGFR-2 antibody (DC101) [72]. This study demonstrated significant antitumour effects of metronomic vinblastine at least in part through antiangiogenic mechanisms in human neuroblastoma xenografts. Furthermore, the antitumour activity of metronomic vinblastine was greatly enhanced by the addition of DC101. The same team went on to demonstrate 2 years later that the combination of metronomic vinblastine or paclitaxel with DC101 was effective in treating multidrug-resistant breast cancer xenografts [73]. Interestingly, this combination showed very little toxicity, unlike the combination of DC101 with metronomic cisplatin or doxorubicin. Therefore, the combination of metronomic MTAs with 'pure' antiangiogenic drugs appeared as a promising strategy in terms of efficacy and low toxicity, right from the very beginning of the field of metronomic chemotherapy. In 2007, Sood and colleagues investigated an interesting combination of taxanes (either paclitaxel or docetaxel) and dual EGFR/VEGFR inhibitor AEE788 in ovarian tumour xenograft models [76]. They found that metronomic taxanes were superior to MTD taxanes in both drug-sensitive and drug-resistant models and synergised with AEE788. Increased antitumour efficacy was associated with decreased tumour angiogenesis, inhibition of EPC mobilisation and reduced tumour-specific cell-free DNA levels. Similarly, the combination of metronomic paclitaxel with anti-EGFR antibody cetuximab was found to induce significant antitumour response in colon cancer xenografts [42]. Consistently with previous studies, metronomic paclitaxel displayed stronger antiangiogenic activity than MTD paclitaxel in this model, which was associated with a differential upregulation of THBS1 expression. A positive interaction was also reported between docetaxel and vandetanib in a mouse model of head and neck cancer [77]. However, in this model, metronomic scheduling of docetaxel administration was associated with severe gastrointestinal toxicity, so that the protocol had to be modified from daily i.p. injection for 28 days to daily injection for 10 consecutive days followed by 9 days of drug-free break. This unexpected morbidity likely due to accumulation of docetaxel in the intraperitoneal cavity prevented definitive conclusions regarding the potential superiority or inferiority of the metronomic protocol compared to the conventional MTD regimen, thus underlining the importance of the route of administration to maximise the benefits of metronomic chemotherapy. Metronomic scheduling of imatinib (Gleevec®) has also been

shown to enhance the sensitivity of neuroblastoma cell lines to vincristine in vitro [78]. Similarly, sorafenib was found to significantly increase the antiangiogenic and antiproliferative effects of low-dose paclitaxel against melanoma cells in vitro [79]. However, the combination of metronomic paclitaxel and sorafenib showed no activity in patients with advanced refractory adrenocortical carcinoma and led to the premature interruption of the phase II trial [80]. This discrepancy highlights the difficulty to predict clinical activity using in vitro experiments and simple in vivo models. For this reason, Kerbel and colleagues recently advocated for the development and implementation of more sophisticated preclinical models, including genetically engineered mouse models, patient-derived xenografts and postsurgical models of either macroscopic or microscopic metastatic disease to better mimic metastatic or adjuvant therapy settings [81, 82].

In the clinic, metronomic chemotherapy is often combined with drug repositioning (i.e. using already approved drugs for new medical applications) in treatment protocols that we called metronomics [83]. In an effort to identify ways to increase the efficacy of chemotherapy, we recently investigated the antiangiogenic and antitumour effects of antihypertensive drugs, β-blockers, alone and in combination with various chemotherapy agents. We thus demonstrated that β-blockers not only displayed potent antiangiogenic properties but were also able to significantly increase the efficacy of chemotherapy, and especially that of MTAs paclitaxel and vincristine, in animal models of breast cancer and neuroblastoma [84, 85]. This strongly suggests that β-blockers could be used in combination with metronomic MTAs in drug-refractory cancers and warrants further investigation.

Overall, the combination of metronomic MTAs and antiangiogenic therapy (i.e. tyrosine kinase inhibitors, antibodies or repositioned drugs) showed promising results in various preclinical models but have not translated into clinical benefits yet.

5.4.2 Metronomic MTAs and Immunotherapy

Another interesting approach consists in combining metronomic chemotherapy and immunotherapy. This strategy is gaining considerable interest from both oncologists and immunologists and has recently met some success in early clinical trials [45]. Although clinical studies undertaken so far have been mostly limited to the use of metronomic cyclophosphamide, a number of preclinical studies have shown some promising results by combining metronomic MTAs and immunotherapy. Thus, Chen et al. showed that metronomic paclitaxel, but not MTD paclitaxel, was synergistic with an antigen-specific DNA vaccine against syngeneic lung cancer models [86]. The combination was associated with increased survival, decreased primary tumour growth and decreased number of metastases. Furthermore, metronomic paclitaxel was associated with increased number of cytotoxic T cells (CD3+ CD8+) in the tumour microenvironment and reduced number of regulatory T cells (CD4+ CD25+ FoxP3+) both in the tumour and the spleen. However, depletion experiments using anti-CD4 and anti-CD8 antibodies revealed that only the cytotoxic T cells were essential to the antitumour effect of the DNA vaccine combined

with paclitaxel. Synergism was also reported between metronomic paclitaxel and specifically engineered peptide mimics from HER-2 and VEGF in several mouse models of human breast cancer [87]. Therefore, although still in its infancy, the combination of metronomic MTAs and immunotherapy appears a very promising strategy that deserves further investigation.

5.5 Future Developments

The two major hurdles to the development and clinical implementation of metronomic protocols based on MTAs are their formulations and bioavailability. Indeed, not only is there only one MTA currently available in oral form (i.e. vinorelbine), but some of the vehicles used in the formulation of these drugs have been associated with significant toxic side effects, especially when used for long periods. This is the case of Cremophor® EL and polysorbate 80, which are used in the commercial formulations of Taxol® and Taxotere®, respectively. Indeed, both these solvents have been associated with adverse events, such as acute hypersensitivity reaction and peripheral neuropathy [88]. Furthermore, preclinical studies have shown that these vehicles significantly hampered the antitumour and antiangiogenic activity of taxanes both in vitro and in vivo [89–91]. Therefore, a significant research effort has been made over the past decade to identify new MTAs and generate new formulations in order to improve efficacy while reducing toxicity.

5.5.1 New MTAs

There are literally hundreds of new compounds with microtubule-targeting properties at various stages of preclinical and clinical development. Here, we will focus on the new MTAs that could potentially be used in metronomic protocols because they either are orally available or have favourable pharmacokinetic profiles.

In the mid-1990s, the discovery of the anticancer and antiangiogenic properties of 2ME2, a natural metabolite of oestradiol devoid of oestrogenic activity, generated some interest in the scientific community [91–95]. 2ME2 binds to β-tubulin near the colchicine-binding site, which results in kinetic stabilisation of microtubule dynamics at low concentration and inhibition of tubulin polymerisation at higher concentrations, subsequently arresting the cell cycle at the G2-M transition [93, 94]. Encouraging results have been reported in clinical trials for the treatment of hormone-refractory prostate cancer [96], multiple myeloma [97] and recurrent and platinum-resistant epithelial ovarian cancer [98] and more recently in patients with metastatic carcinoid tumours where 2ME2 was used in combination with bevacizumab [99]. The advantage of 2ME2 over other MTAs is that it is not a substrate of multidrug resistance pumps [100] and it does not induce neurotoxicity or myelosuppression in cancer patients [96, 97, 101]. However, most clinical trials also revealed that the bioavailability of 2ME2 was a limiting factor [96, 97, 102]. Therefore, new analogues of 2ME2 have been developed in order to improve its pharmacokinetic

profile. We and others have characterised the anticancer and anti-vascular properties of 2ME2 analogue, ENMD-1198 [9, 33, 103, 104]. We showed that ENMD-1198 was able to inhibit most endothelial cell functions involved in tumour angiogenesis: motility, chemotaxis, proliferation and morphological differentiation into vascular structures [9]. We also found that this compound displayed vascular-disrupting properties, via extensive microtubule depolymerisation, accumulation of actin stress fibres and large focal adhesions. Finally, we demonstrated that ENMD-1198, and to a lesser extent 2ME2, induced a decrease in VEGFR-2 expression in endothelial cells [9], while Moser et al. showed that this compound could disrupt hypoxia signalling in hepatocellular carcinoma cells, by preventing the accumulation of HIF-1α under hypoxic conditions [33]. Collectively these studies revealed that ENMD-1198 can affect tumour angiogenesis at two different levels: at the tumour level by blocking HIF-1α signalling in cancer cells and at the endothelium level by blocking endothelial cell functions involved in angiogenesis and by decreasing VEGFR-2 expression.

Elsewhere, Aneja and colleagues have investigated the antitumour, antiangiogenic and vascular-disrupting properties of a novel orally available MTA, EM011 [105–108]. This noscapine analogue is able to interfere with the proliferation, migration, invasion and morphological differentiation of endothelial cells into capillary tubes in vitro and inhibit tumour angiogenesis in vivo. Interestingly, EM011 was shown to exert significant antitumour effects at doses that were not immunosuppressive. Furthermore, EM011 was also able to disrupt hypoxia signalling, by inducing the proteasome-dependent and VHL-independent degradation of HIF-1α in prostate cancer cells. Similarly, a number of new MTAs have been shown to target HIF-1α, including MPT0B098 and ELR510444. MPT0B098 is a novel indoline-sulfonamide compound with microtubule-depolymerising properties that can not only inhibit HIF-1α protein expression but also destabilise HIF-1α mRNA by decreasing the translocation of RNA-binding protein, HuR, from the nucleus to the cytoplasm [109]. ELR510444 is another orally available MTA that binds to the colchicine-binding site of β-tubulin, decreases HIF-1α and HIF-2α levels through VHL-dependent mechanisms and induces significant antitumour effects in breast cancer and renal cell carcinoma models [110, 111].

Finally, although not metronomic agents per se, a considerable effort has been put recently to develop novel orally available vascular-disrupting agents as an alternative to combretastatins. This is the case of CYT997, which was developed by Wilks and colleagues and has reached clinical trials [112–115], and BNC105, developed by Flynn and colleagues and currently investigated in a phase I/II trial in ovarian cancer patients in combination with carboplatin and gemcitabine (NCT01624493) [116–118].

5.5.2 New Drug Formulations

Besides the identification of novel MTAs, a lot of effort has been put recently into developing novel formulations for this class of chemotherapeutic drugs. The

objectives of these new formulations are to reduce toxicity, improve bioavailability and increase tumour targeting and/or selectivity.

As early as the mid-1990s, researchers have worked on developing new paclitaxel formulations in order to improve its bioavailability and prevent hypersensitivity reactions due to Cremophor® EL [119]. Soon-Shiong and Desai thus developed ABI-007, a nanoparticle albumin-bound formulation of paclitaxel also called nab-paclitaxel, which was approved by the FDA in 2005 under the name Abraxane® for the treatment of drug-refractory or relapsed breast cancer. This formulation of paclitaxel may also prove beneficial for the treatment of other cancers, such as melanoma, lung and pancreatic cancer [120], and more than 100 clinical trials are currently underway. Interestingly, a randomised phase III clinical trial recently showed very encouraging results using a combination of nab-paclitaxel and gemcitabine for the treatment of pancreatic cancer [121]. Despite showing improved efficacy and reduced toxicity as compared to Taxol® in clinical studies [122], Abraxane® has not completely replaced its parental compound, most likely due to its very high cost. Furthermore, this formulation is not orally available and therefore not suitable for metronomic scheduling, despite showing promising results in preclinical models [90]. In this regard, the recent development of an oral solid dispersion formulation of paclitaxel could open major avenues for paclitaxel-based metronomic protocols [123]. Indeed, the ModraPac001 formulation showed clinically relevant systemic exposure to paclitaxel in patients with advanced cancer and present attractive characteristics for oral metronomic chemotherapy with neutral taste, dosing accuracy and 2-year ambient shelf life.

Other strategies undertaken to improve the pharmacokinetics and/or targeting of MTAs include liposome encapsulation, PEG- and/or peptide-conjugated nanoparticles, hyaluronic acid conjugates and liquid crystal nanoparticles. Dellian and colleagues used cationic liposomes to encapsulate paclitaxel and increase its neovascular targeting [124–126]. EndoTAG-1, also formerly known as LipoPac, has shown increased anti-vascular and antitumour activity in preclinical models of melanoma and lung and pancreatic cancer. Importantly, stronger antitumour effects were observed when EndoTAG-1 was administered following a metronomic protocol rather than at the MTD [126]. Recently, EndoTAG-1 was found to be safe and showed encouraging results in early clinical trials in patients with advanced pancreatic and head and neck cancers [127, 128]. Elsewhere, Zhang and colleagues have used peptide-modified micelles to encapsulate docetaxel as well as uncoupled and peptide-modified sterically stabilised liposomes to encapsulate paclitaxel [129, 130, 131]. They showed increased antiangiogenic and/or antitumour activity in vitro and in vivo in breast cancer and fibrosarcoma xenografts as compared to classical formulations of docetaxel and paclitaxel. Interestingly, they conjugated their micelles and liposomes with NGR peptides (Asn-Gly-Arg) to target CD13/aminopeptidase N, a membrane-bound exopeptidase selectively expressed by tumour endothelial cells and a number of solid tumours [132]. Yu et al. used K237 (HTMYYHHYQHHL) peptide-conjugated nanoparticles to encapsulate paclitaxel and specifically target VEGFR-2, which resulted in increased antiangiogenic activity in vitro and in vivo in breast cancer xenografts as compared to Taxol® [133]. An

alternative strategy consists in using hyaluronic acid conjugation to target the cell-surface glycoprotein CD44, which is expressed by a large number of tumours and by tumour endothelial cells [134]. Sood and colleagues compared the antitumour effects of hyaluronic acid conjugates of paclitaxel administered at the MTD or following a metronomic schedule [134]. They found that metronomic dosing showed superior antiangiogenic and antitumour activity in both chemosensitive and multidrug-resistant ovarian cancer models. Interestingly, the antiangiogenic effect of metronomic hyaluronic acid-paclitaxel was associated with substantial increases in THBS1 expression. Different approaches have also been successfully used to specifically target docetaxel to prostate cancer cells, taking advantage of the selective expression of the prostate-specific membrane antigen (PSMA). These include conjugating nanoparticles with RNA aptamers that specifically recognise the extracellular domain of PSMA [135] or with urea-based small-molecule peptidomimetic inhibitor of PSMA [136].

Finally, one of the most recent developments in drug delivery systems that may have direct application in metronomic chemotherapy is the utilisation of liquid nanocrystals. Cervin et al. thus developed a novel liquid crystal nanoparticle formulation of docetaxel and showed its superior antitumour activity in a prostate cancer xenograft model as compared to Taxotere® [91]. The new formulation also displayed interesting advantages in terms of increased tolerability and longer shelf life. A similar approach was also used to improve the bioavailability of 2ME2. However, the results of two phase II trials in metastatic prostate cancer and metastatic renal cell carcinoma using oral nanocrystal dispersion of 2ME2 showed a lack of clinical efficacy and were both prematurely terminated [137, 138]. Additional trials have been performed using the same formulation of 2ME2 in patients with recurrent or resistant ovarian cancer (NCT00400348) and glioblastoma (NCT00481455), but results were not reported, suggesting a lack of efficacy in these settings as well.

Overall, there has been a lot of promising developments at the preclinical level in the field of MTAs in recent years, both in terms of new compounds and novel formulations. However, these innovations are yet to translate into effective metronomic chemotherapy protocols in the clinic.

Conclusions

MTAs are amongst the most successful chemotherapeutic drugs and are part of the standard of care for a wide variety of human malignancies. The discovery of their potent anti-vascular properties has contributed to the evolution of their administration schedules from very high dose injected once every 3 weeks to lower doses administered weekly and even lower doses in metronomic protocols. The key role played by microtubules in angiogenesis explains the unique antiangiogenic properties of MTAs. Although metronomic MTAs have shown very promising activity in preclinical studies either alone or in combination with antiangiogenic agents and immunotherapy, the lack of reliable biomarkers and oral formulations of MTAs still hampers the widespread implementation of MTA-based metronomic chemotherapy in the clinic. Advanced medicinal chemistry and the use of nanotechnology have recently led to the development of new

MTAs and novel oral formulations with improved bioavailability. Future preclinical studies will need to determine how to best combine these new agents with immunotherapy, targeted and/or repositioned drugs and how to select patients likely to benefit from these combination treatments. Innovative clinical trials will then be warranted to validate the findings and implement novel therapeutic options for cancer patients.

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Part II

Clinical Activity of Metronomic Chemotherapy

Metronomic Chemotherapy in Breast Cancers

6

Elisabetta Munzone, Francesco Bertolini, and Marco Colleoni

Abstract

Breast cancer is a common disease in women and its incidence is increasing. A proportion of breast cancer patients are metastatic at diagnosis or become metastatic during the follow-up period and need a personalized and/or target treatment approach. Metronomic chemotherapy can be regarded as a multi-targeted therapy for advanced disease, combining effects not only on tumor cells but also on their microenvironment by inhibiting tumor angiogenesis, stimulating anticancer immune response, and potentially inducing tumor dormancy. In the last 10 years, many phase I/II trials with metronomic chemotherapy in advanced breast cancer were published and will be described in details. Although this treatment approach was initially designed to maintain a stable disease as long as possible for metastatic patients that cannot be cured, as results become evident, researchers and clinicians started looking for new applications of this therapeutic strategy. Biomarkers are being developed to identify reliable surrogate markers of response and also to identify the proper patients to be treated. Nowadays, there are several ongoing trials to identify the optimal regimen and schedule of metronomic chemotherapy in the different settings of breast cancer patients. Most trials are aimed at patients with triple negative disease, because in this setting chemotherapy still represents one of the most reliable option. Finally, the potential development of metronomic chemotherapy in breast cancer is still a matter of research with particular attention to identify biomarkers and individual tumor characteristics that can better address the use of this treatment strategy in the future.

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6.1 Introduction

Breast cancer is one of the most prevalent malignancies in women in almost all countries. In Europe, women have an 8 % chance to suffer from breast cancer before the age of 75 and a 2 % chance of dying from the disease [1]. Although in the last 20 years the incidence of breast cancer has increased 1.5 % annually, the mortality rates have been progressively decreasing.

Approximately 5–10 % of newly diagnosed breast cancer patients are metastatic at diagnosis, and nearly 10–15 % of breast cancer patients become metastatic in the first 3 years after diagnosis. Nevertheless, a proportion of patients are also likely to develop metastases 10 years or even later after first detection [2].

Metastatic breast cancer remains an incurable but treatable disease. A key component of the approach to this disease is conventional chemotherapy and/or endocrine therapy according to breast cancer biology. In recent years, targeted therapies have been added to various chemotherapy backbones. The median survival rate of the patient with metastasis is within the range of 3 years.

The importance of understanding the mechanisms underlying the metastatic process and the complex interactions between tumor and host during disease progression has been widely recognized. Nevertheless, despite multidisciplinary approaches and novel target treatments, metastatic disease remains the primary cause of death in the majority of patients with breast cancer.

In recent years, clinicians increasingly agree in considering breast cancer not only as one disease; in fact models of breast cancer as a systemic and heterogeneous disease suggest novel ways to target the process of metastasis.

6.1.1 Breast Cancer Is Not One Disease: Subtypes and Heterogeneity

During the last decade, research has focused in depth on the molecular biology of breast cancer. Particularly, high-throughput approaches allowed researchers to ascertain the nature of breast cancer revealing that this disease is characterized by the interconnection of several signaling pathways. Both the cellular microenvironment and the innate characteristics of the patient might influence pathophysiologic characteristics of breast cancer, its outcome, and treatment response.

These findings led researchers to understand that each patient entails a particular case where personalized medicine could play a crucial role. Clinicians are increasingly seeking to propose a personalized medicine approach, but there are still many unresolved issues to be addressed.

Especially in those individuals who lack a clear therapeutic target, there is a special need to identify and validate new molecular markers. In fact, even if a tumor has a specific druggable pathway, tumor cells often display an unexpected resistance that allows them to escape death.

The hierarchical cluster analysis initially performed by Perou et al. revealed four molecular subtypes: luminal, HER2, basal like, and normal breast [3]. The subsequent expansion of this work in a larger cohort of patients showed that the luminal

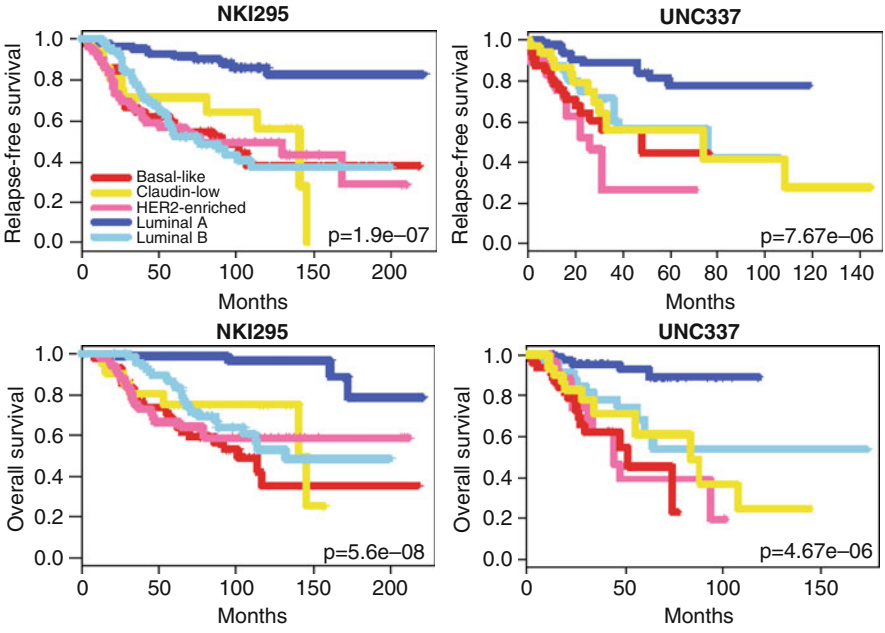

Fig. 6.1 Survival data of the different molecular subtypes (Adapted from Prat et al. [37])

subgroup could be divided into at least two groups (luminal A and B) and that different molecular subtypes were associated with different prognoses (Fig. 6.1) [4]. This new classification validated by independent groups was based on an unsupervised analysis, grouping tumors according to their biological characteristics regardless of their clinical or prognostic variables.

These molecular classified subgroups correspond reasonably well to clinical-pathological characterization on the basis of estrogen receptor (ER) and HER2 status, as well as proliferation markers or histological grade performed by means of immunohistochemistry (IHC) techniques [5, 6].

During the 2011 St Gallen Consensus Conference, a surrogate definition of intrinsic subtypes of breast cancer was issued for purposes of clinical use [7], and it was further refined in the recent 2013 Consensus Conference [8].

The molecular heterogeneity of breast cancer is reflected in the clinical course of the disease and in responses to treatment.

Recently, powerful gene-sequencing techniques have revealed many genetic and epigenetic alterations governing breast cancer [9–14]. The existence of extended genetic variation within a single tumor mass is called intratumoral heterogeneity [15], and new data support the idea that tumor heterogeneity represents a branching pattern of tumor evolution, as opposed to the traditionally accepted linear model. Metastatic disease represents the final stage of this branched tumor evolution [16]. Therefore, in the setting of metastatic breast cancer, this heterogeneity has direct consequences for the emergence of therapy resistance even to targeted agents.

6.1.2 The Role of Metronomic Chemotherapy

Metronomic chemotherapy exerts both direct and indirect effects not only on tumor cells but also on their microenvironment by inhibiting tumor angiogenesis and stimulating anticancer immune response. In addition, metronomic chemotherapy can directly affect tumor cells through a theoretical drug-driven dependency/deprivation effect and can exert additional anticancer effects and potential (re)induction of tumor dormancy.

New mechanisms have been identified, such as the restoration of the anticancer effect of the immune system. Therefore, metronomic chemotherapy can be regarded as a multi-targeted therapy [17]. Although the rationale of metronomic chemotherapy is yet to be fully elucidated, the use of low-dose oral chemotherapy in the clinic has been initially restricted to palliative purposes.

However, after the publication of several phase I/II trials in metastatic breast cancer (Table 6.1), clinicians are now more inclined to give credit to metronomic chemotherapy.

6.2 Metronomic Regimens in Breast Cancer: Early Trials

Among conventional cytotoxic agents that may exert a tumor suppressive effect through an antiangiogenic mechanism, cyclophosphamide (CTX) and methotrexate (MTX) were those with a significant bioavailability and were therefore the best candidate for a metronomic regimen to treat advanced breast cancer patients. The first trial in this setting was reported in 2002 in a series of 63 advanced and pretreated breast cancer patients who received metronomic CM (CTX 50 mg/day administered continuously and MTX 2.5 mg bid on days 1 and 2 each week). In this patients' population, an overall response rate of 19 % (95 % CI 10.2–30.9 %) and an overall clinical benefit (CR + PR + stable disease ≥24 weeks) of 31.7 % (95 % CI 20.6–44.7 %) were reported without significant toxicity [18].

Few years later, the same authors reported results and long-term follow-up for patients with metastatic breast carcinoma who obtained prolonged clinical benefit with CM. One hundred and fifty-three patients who achieved prolonged clinical benefit for a duration of 12 months or more were considered for the analysis. The proportion of patients who achieved prolonged clinical benefit was 15.7 % (95 % confidence interval 9.9–21.4 %). Median time to progression for patients with prolonged clinical benefit was 21 months (range 12–37+ months) [19].

To improve results obtained, a subsequent trial in a similar population of patients investigated the association of metronomic CM with thalidomide [20]. Thalidomide is a derivative of glutamic acid and has immune-modulating activity secondary to inhibition of lymphocyte proliferation [38]. The drug is also able to inhibit tissue tumor necrosis factor-α production by stimulating human monocytes and lymphocytes [39, 40]. In addition to its immune-modulatory activities, oral thalidomide can inhibit angiogenesis induced by basic fibroblast growth factor and vascular endothelial growth factor (VEGF) in the rabbit corneal micropocket assay [41, 42].

Table 6.1 Published trials with metronomic chemotherapy in metastatic breast cancer

	Schedule	Phase	*N.* pts	Pretreatment (Y/N)	ORR % (95 % CI range) CB % (95 % CI range)	Median TTP	Refs.
Early trials	CM: CTX 50 mg/day MTX 2.5 mg bid (d1, 2/week)	II	63 153 (CB ≥12 months)	Y	ORR: 19 % (10.2–30.9 %) CB: 31.7 % (20.6–44.7 %) CB >12 months: 15.7 %	21 months	[18, 19]
	CM ± thalidomide (200 mg/day)	II randomized	171	Y	ORR arm A: 20.9 % (12.9–31 %) ORR arm B: 11.8 % (5.8–20.6 %) CB 41.5 % (34–49.3 %)	Arm A: 3.8 months Arm B: 4.1 months	[20]
	CM + dalteparin 5,000 UI/day + PDN 5 mg/day (dalCMP)	I/II	41	Y (61 %)	ORR 17 % (7–32 %) CB 24 % (12–40 %).	10 weeks (95 % CI, 8–17 weeks)	[21]
Metronomic chemotherapy with target/antiangiogenic therapy	CM + trastuzumab (6 mg/kg/3 weeks)	II	22	Y	CB 46 %	6 months	[22]
	CM ± bevacizumab (10 mg/kg q2w)	II randomized	42	Y	ORR 41 %	NR	[23]
	Bevacizumab 10 mg/kg q2w CTX 50 mg/day Capecitabine 500 mg tid	II	46	Y (31 %)	ORR 48 % (33–63 %) CB 68 % (51–81 %)	42 weeks	[24]
	Bevacizumab 15 mg/kg q3w CTX 50 mg/day Capecitabine 500 mg tid Erlotinib 100 mg/day	II	26	N	ORR 62 % (41–81 %) CB 75 % (53–90 %)	43 weeks	[25]
	CM + bevacizumab 10 mg/kg q2w [trastuzumab in HER-2 pos]	II	22	Y	ORR 31.8 % (13.9–54.9 %) CB 63.6 % (40.7–82.8 %)	7.5 months (HER2pos vs. neg)	[26]
	CM + vandetanib (V) V: C1, 100 mg; C2, 200 mg; C3, 300 mg	I	23	Y (92 %)	ORR 10 % (1–32 %)	NR	[27]

(continued)

Table 6.1 (continued)

	Schedule	Phase	*N.* pts	Pretreatment (Y/N)	ORR % (95 % CI range) CB % (95 % CI range)	Median TTP	Refs.
New uses for old drugs	CTX 65 mg/m^2/day dd 1–14 capecitabine 1,000 mg/m^2 bid dd 1–14	II	66		ORR 30.3 % (20–43 %) CB 53 % (38–62 %)	5.2 months (95 % CI, 4.2–6.2)	[28]
	PLD 20 mg/m^2 q2w + CTX 50 mg day	II	29	N (loc adv)	ORR 62.1 % (42.4–78.7 %)	NR	[29]
	PLD 20 mg/m^2 q2w	Retrospective	52	Y	ORR 18 % (8.2–32.7 %) CB 45 % (30.3–59.7 %)	4.2 months (95 % CI 3.4–6.2)	[30]
	VNB 70 mg/m^2 days,3,5 (1 week on/1 week off)	II	34		ORR: 38 % (28–48 %) CB: 68 % (60.7–81.9 %)	7.7 months (95 % CI, 6.9–9.05)	[31]
	VNB (30 → 60 mg Cape 800 → 1,250 mg/m^2/day	I/II	36	Y (67 %)	ORR 33 %	5.6 months (95 % CI: 2.8–8.4)	[32]
	TMZ 75 mg/m^2 + WBRT → VNB 70 mg/m^2 days 3,5+TMZ 75 mg/m^2 d1–21	II	36		ORR 52 % (38–67 %) CB: 77 % (62.7–88.9 %)	8 months (95 % CI, 6.8–8.7 months)	[33]
Metro CT with endocrine therapy	CM + fulvestrant 250 mg i.m. q 4 weeks	Retrospective	33	Y	CB 56 % (38–74 %)	4.7 months (95% CI 3.6–6.7)	[34]
	Letrozole ± CTX 50 mg/day	II randomized	114	N T2–4 N0–1	ORR 71.9 % (60–83.8 %) ORR 87.7 % (78.6–96.2 %)	NR	[35]
	CTX + megestrol acetate (80 mg/day)	II	29	Y	ORR 31 %	7.4 months (3.8–10.88)	[36]

Abbreviations: *CTX* cyclophosphamide, *MTX* methotrexate, *PDN* prednisone, *PLD* pegylated liposomal doxorubicin, *VNB* vinorelbine, *TMZ* temozolomide, *WBRT* whole-brain radiotherapy, *ORR* overall response rate, *CB* clinical benefit, *TTP* time to progression, *NR* not reported

Therefore, the activity and biological effects of low-dose oral CTX and MTX was compared with the same combination plus thalidomide. Overall, 171 patients with advanced breast cancer were randomized to receive oral CM or the same regimen plus thalidomide (200 mg daily). Nevertheless, the addition of thalidomide did not improve results previously obtained with the CM regimen.

Preclinical evidence showed a synergism between metronomic chemotherapy and antiangiogenic agents. Moreover, corticosteroids and low-molecular-weight heparins have known antiangiogenic properties [43–45]; therefore, a phase I/II trial combining daily dalteparin, cyclophosphamide, twice-weekly methotrexate, and daily prednisone (dalCMP) was conducted in metastatic breast cancer [21] accruing 41 patients. Median time to progression (TTP) was 10 weeks (95 % CI, 8–17 weeks), and median OS was 48 weeks (95 % CI, 32–79 weeks). VEGF levels decreased but not significantly, whereas sVEGFR-1 and sVEGFR-2 levels increased significantly after 2 weeks of therapy. Authors concluded that metronomic dalCMP was a safe, well-tolerated, and clinically active treatment in this setting of patients.

6.3 Combining Metronomic Chemotherapy with Molecularly Target and Antiangiogenic Target Agents

In order to explore the activity of metronomic chemotherapy plus targeted therapy, 22 patients with metastatic breast cancer and with the overexpression or amplification of HER2-/neu were treated with trastuzumab (6 mg/kg every 3 weeks) in combination with metronomic CM [22]. All patients were already pretreated with trastuzumab plus other cytotoxics. The clinical benefit calculated in all patients and in those with disease resistant to previous trastuzumab therapy was 46 % (95 % CI, 24–68 %) and 27 % (95 % CI, 6–61 %), respectively. Median time to progression was 6 months and median duration of treatment was 5 months (range, 0.7–18.4 months, and range, 1–18 months, respectively). These results showed that the combination of trastuzumab and metronomic chemotherapy is effective and minimally toxic in pretreated advanced breast cancer patients.

There is a rationale for the combination of metronomic chemotherapy and targeted antiangiogenic agents like bevacizumab. In preclinical models, the combination of metronomic chemotherapy with a VEGFR2 antibody resulted in sustained regressions of large tumors, without overt toxicity occurring during the course of treatment [46]. In a randomized phase II trial comparing metronomic CTX and MTX with the same regimen plus bevacizumab in women with pretreated advanced breast cancer, a planned interim analysis after the first 19 patients per arm revealed a significant advantage in favor of the combined arm in terms of objective response (41 %) [23].

Given these premises, 46 patients with advanced breast cancer received metronomic oral capecitabine (500 mg thrice daily) and cyclophosphamide (50 mg daily) plus bevacizumab (10 mg/kg every 2 weeks) within a phase II trial [24]. The overall response rate was 48 % (95 % CI, 33–63 %); long-term disease stabilization

(SD ≥24 weeks) occurred in eight patients, for an overall clinical benefit of 68 % (95 % CI, 51–81 %). Median time to progression was 42 weeks (95 % CI, 26–72 weeks). Treatment with metronomic capecitabine and cyclophosphamide in combination with bevacizumab was effective and was minimally toxic. The number of baseline circulating endothelial cells (CECs) significantly correlated with response and outcome, therefore supporting further studies on this surrogate marker for the selection of patients to be candidates for antiangiogenic treatments.

A subsequent trial in the same patient population had the aim to determine the safety and efficacy of metronomic chemotherapy combined with targeted drugs such as bevacizumab and erlotinib [25]. Twenty-six untreated patients with HER2-negative metastatic breast cancer and poor hormone receptor expression received metronomic oral capecitabine (500 mg thrice daily) and cyclophosphamide (50 mg daily) plus bevacizumab (15 mg/kg every 3 weeks) and erlotinib (100 mg daily). The overall clinical benefit was 75 % (95 % CI, 53–90 %). Median time to progression was 43 weeks (95 % CI, 21–69). Patients with low levels of circulating endothelial progenitors (CEPs) at baseline had a significantly improved progression-free survival. Toxicity was generally mild. The analysis of the results suggested that the metronomic chemotherapy combined with bevacizumab and erlotinib is effective and well tolerated in a group of HER2-negative, estrogen receptor, and progesterone receptor-poor advanced breast cancer.

A similar trial explored the activity of cyclophosphamide 50 mg p.o. daily, methotrexate 1 mg/kg i.v. every 14 days, and bevacizumab 10 mg/kg i.v. every 14 days in an anthracycline and taxane refractory metastatic breast cancer patient population [26]. Trastuzumab was added in HER2-overexpressing tumors. Among the 22 patients evaluable for response, the clinical benefit was 63.6 % (95 % CI 40.7–82.8 %). Median progression-free survival was 7.5 months; overall survival was 13.6 months. HER2-overexpressing or high proliferative index tumors had better 6-month PFS (75 % vs. 34 % in HER2-negative tumors, $p=0.043$; 67 % vs. 0 % in Ki-67 ≥20 % tumors, $p=0.015$).

A recently published phase I study evaluated the safety and tolerability of antiangiogenic therapy using vandetanib and metronomic cyclophosphamide and methotrexate in metastatic breast cancer [27]. Vandetanib is an oral once daily administered inhibitor of VEGFR, EGFR, and RET signaling with activity in combination with chemotherapy in some solid tumors [47]. Twenty-three patients with 0–4 prior chemotherapy regimens were treated. All patients received cyclophosphamide 50 mg daily, methotrexate 2.5 mg days 1–2 weekly, and vandetanib daily in 3 dose-escalation cohorts: 100 mg (C1), 200 mg (C2), and 300 mg (C3). Toxicities were mild and included nausea, vomiting, fatigue, and rash. In all cohorts, a third of patients required vandetanib dose reduction. Of the 20 response-evaluable patients, 10 % had a partial response and 15 % stable disease ≥24 weeks. Proteomic analyses demonstrated changes in platelet content of angiogenesis regulators, including vascular endothelial growth factor and platelet factor 4, with exposure to therapy. This regimen was tolerable at a maximum vandetanib dose of 200 mg; modest clinical activity was observed in this heavily pretreated population. Changes observed in the platelet proteome have been supposed to serve as pharmacodynamic markers of angiogenesis inhibition.

6.4 New Uses for Old Drugs

Metronomic cyclophosphamide (CTX) and capecitabine may have a greater potential for treatment of metastatic breast cancer, because of their antiangiogenic activity resulting from the metronomic dosage and upregulation of thymidine phosphorylase by CTX.

Therefore, a phase II trial was conducted in metastatic breast cancer patients receiving oral metronomic CTX 65 mg/m^2 daily on days 1–14 plus capecitabine 1,000 mg/m^2 twice daily on days 1–14 [28]. The treatment was repeated every 3 weeks. Sixty-six patients were evaluated for efficacy, and after a median follow-up time of 26 months, the median time to progression was 5.2 months (95 % CI, 4.2–6.2 months), and the median overall survival was 16.9 months. The overall response rate was 30.3 % (95 % CI, 20–43 %). Clinical benefit rate was 53.0 % (95 % CI, 38–62 %).

Caelyx is a pegylated liposomal doxorubicin (PLD), used as a single agent in advanced breast cancer at conventional doses ranging from 40 to 50 mg/m^2 every 3–4 weeks, with objective response rates ranging from 31 to 33 % [48].

The pharmacokinetics of PLD supports the rationale for using the drug in a metronomic fashion, mainly because of the polyethylene-glycol-coated liposomic coat surrounding the molecule. Liposomes markedly prolong circulation and enhance drug accumulation inside the tumor, retarding uptake by mononuclear phagocytes; PLD achieves a longer half-life than non-pegylated liposomal doxorubicin, as the polyethylene glycol liposome interacts with plasma proteins and inhibits mononuclear phagocytes, consequently prolonging circulation time [49]. The drug is also characterized by a reduced volume of distribution, a long intravascular circulating half-life, and a slow plasma clearance compared with free doxorubicin.

The activity and safety of intravenous PLD 20 mg/m^2 biweekly for eight courses in combination with metronomic cyclophosphamide 50 mg/day orally were evaluated in 29 patients with locally advanced breast cancer who were not suitable to receive a standard chemotherapy due to age or comorbidities or who asked for a regimen with low incidence of toxic effects irrespective of age [29]. Eighteen patients (62.1 %) achieved a partial response (including one pathological complete response), ten patients (34.5 %) achieved a stable disease, and one patient experienced a progressive disease. Treatment was well tolerated, with no grade 4 toxicities, and with grade 3 skin toxicity in three patients and hand-foot syndrome in four patients. The rate of breast-conserving surgery was 44.8 %. Although the regimen was well tolerated, this combination chemotherapy showed a limited activity in the preoperative setting.

In a case series report carried out in both anthracycline-naive and pretreated metastatic breast cancer patients, feasibility, clinical efficacy, and tolerability of PLD administered with a the metronomic schedule of 20 mg/m^2 i.v. every 2 weeks were tested [30].

Among 52 patients enrolled in the trial, 44 patients were assessed for either response or toxicity. Eight patients (18 %) had partial responses and 17 (39 %)

stable disease, with a clinical benefit of 45 % (95 % CI: 30.3–59.7 %). Nineteen patients (43 %) had progressive disease. Neither grade 3 nor grade 4 hematological or clinical side effects were recorded, except for two patients with grade 3 palmar-plantar erythrodysesthesia. No cardiac toxicity was recorded. Metronomic administration of PLD resulted as a feasible and active treatment for extensively pretreated metastatic breast cancer patients, alternative to classic anthracyclines. Overall, a good balancing of clinical efficacy with a good quality of life was reached in terms of reduced side effects and low personal costs for the patient.

Anti-tubulin agents are known to have antiangiogenic effects at doses below that required to induce cytotoxicity, including taxanes, such as paclitaxel [50] and docetaxel [51], and vinca alkaloids such as vinblastine [52].

Most studies evaluating metronomic scheduling of anti-tubulin agents have used weekly drug scheduling. Given the availability of an oral formulation of vinorelbine, which has an oral bioavailability of 43 % and a terminal half-life of approximately 29 h (±7.9 h), permitting more thrice weekly or every other day dosing [53, 54], metronomic oral vinorelbine trials were conducted in a series of patients with advanced breast cancer.

Phase I trials of metronomic oral vinorelbine in patients with advanced cancer indicated that 50 mg given three times a week is the optimal dose for a metronomic schedule, yielding sustainable antitumor activity without overt toxicity [55–57].

Oral vinorelbine at 70 mg/m^2, fractionated on days 1, 3, and 5, for 3 weeks on and 1 week off, every 4 weeks was administered to 34 elderly patients with metastatic breast cancer (median age, 74 years; range, 70–84 years) [31]. Patients were treated with for a maximum of 12 cycles. The objective response rate was the primary end point. Two patients achieved complete responses (6 %) and 11 achieved partial responses (32 %). Median progression-free survival and median overall survival were 7.7 months (95 % confidence interval, 6.9–9.05 months) and 15.9 months (95 % CI, 13.1–15.91 months), respectively, for all patients. The fractionated administration of oral vinorelbine is well tolerated with promising activity in elderly metastatic breast cancer patients.

In a recently published trial, escalated doses of oral metronomic vinorelbine (starting dose 30 mg) every other day continuously and capecitabine (starting dose 800 mg/m^2 bid) on days 1–14 every 21 days were administered [32]. Thirty-six women were enrolled at eight escalating dose levels. For 24 patients, treatment was first line, for 8 second line, and for 4 third line. The dose-limiting toxicity (DLT) level was reached at oral metronomic vinorelbine 70 mg and capecitabine 1250 mg/m^2, and the recommended maximum tolerated doses (MTD) were vinorelbine 60 mg and capecitabine 1,250 mg/m^2. DLTs were febrile neutropenia grades 3 and 4, diarrhea grade 4, and treatment delays due to unresolved neutropenia. There was no treatment-related death. The main toxicities were grade 2–3 neutropenia in 16.6 % of patients each, grade 2–3 anemia 16.5 %, grade 2–4 fatigue 27.5 %, grade 2–3 nausea/vomiting 11 %, and grade 3–4 diarrhea 8.2 %. Two complete and ten partial responses were documented. Therefore, oral metronomic vinorelbine with capecitabine was deemed as a well-tolerated and feasible regimen that merits further evaluation in this patients' setting.

The role of metronomic chemotherapy was further explored in the setting of patients with brain metastases from breast cancer [33]. Thirty-six patients with newly diagnosed brain metastases were treated with temozolomide (TMZ) orally administered at a dose of 75 mg/m^2 during whole-brain radiotherapy, followed by 4 weeks off-therapy and a subsequent administration of oral 70 mg/m^2 vinorelbine fractionated in days 1, 3, and 5, weekly for 3 consecutive weeks plus TMZ at 75 mg/m^2 on days 1–21. Cycles were repeated every 4 weeks for up to 12 additional cycles. The primary end point was the evaluation of the objective response rate (ORR). Three complete responses and 16 partial responses have been achieved with an ORR of 52 % (95 % CI 38–67 %) that exceeded the target activity per study design. The median progression-free survival and overall survival were 8 and 11 months, respectively. The schedule appeared to be well tolerated, and side effects reported were generally mild. Authors concluded that the treatment was safe and a significant number of objective responses were observed with a significant improvement in quality of life in this particular setting of breast cancer patients.

6.5 Combining Metronomic Chemotherapy with Endocrine Therapy

The activity of oral metronomic CM combined with fulvestrant was retrospectively assessed in two cohorts of heavily pretreated estrogen receptor-positive advanced breast cancer patients [34]. A series of 33 postmenopausal patients received fulvestrant 250 mg via i.m. injection every 28 days. Twenty patients in the first cohort added metronomic CM after disease progression, continuing fulvestrant at the same dose. Thirteen patients in the second cohort started fulvestrant plus metronomic CM upfront. Clinical benefit for both cohorts was 56 % (95 % CI 38–74 %). The addition of metronomic CM did not determine relevant toxicities. Treatment with fulvestrant plus metronomic CM was effective in this group of patients and was minimally toxic providing long-term disease control in a high proportion of them.

To investigate the activity of letrozole with or without oral metronomic cyclophosphamide as primary systemic treatment in elderly breast cancer patients, 114 consecutive elderly women with T2–4 N0–1 and estrogen receptor-positive breast cancer were randomly assigned to primary letrozole therapy (2.5 mg daily for 6 months) or a combination of letrozole plus oral cyclophosphamide (50 mg/daily for 6 months) in an open-labeled, randomized phase II trial [35]. Overall response rate was 71.9 % (95 % CI, 60.0–83.8) in the 57 patients randomly assigned to receive primary letrozole and 87.7 % (95 % CI, 78.6–96.2) in the 57 patients randomly assigned to receive letrozole plus cyclophosphamide.

The safety and antitumor activity of the metronomic chemo-hormonal therapy with daily cyclophosphamide and twice daily megestrol acetate (mCM regimen) were investigated in patients with metastatic pretreated breast cancer [36]. This phase II study enrolled 29 pretreated postmenopausal patients with multiple metastatic sites. Four patients had a triple negative status, 19 a positive hormonal ER and PgR status, and 3 HER-2 overexpression. Patients received treatment with

cyclophosphamide (50 mg/daily day 1–21/q28) and fractionated megestrol acetate (80 mg twice a day). The overall objective response rate was 31.0 %, disease control rate 41.3 %, mean time to tumor progression 7.4 months (CI 95 %, 3.8–10.88, range 1–48 months), and mean overall survival 13.4 months (CI 95 %, 7.24–17.18, range 1–53 months).

6.6 Tolerability of Metronomic Chemotherapy

Despite patients treated within clinical trials of metronomic chemotherapy are often heavily pretreated or elderly, toxicities and long-term effects reported in the majority of trials showed that metronomic chemotherapy, alone or in combination, is generally well tolerated.

High-grade toxic effects were either rare or absent, and the most common toxic effects were grade 1 nausea and/or vomiting, grade 1 and 2 anemia, neutropenia, leucopenia, as well as low-grade fatigue. Alopecia grade 1 was rarely reported.

Some toxic effects were observed when metronomic chemotherapy was combined with other agents, such as bevacizumab, or when combined with standard doses of chemotherapy.

Nevertheless, clinicians should bear in mind that prolonged metronomic chemotherapy may lead to high total cumulated doses of anticancer agents, which can be associated with secondary diseases. For instance, high cumulated dose of etoposide [58] or temozolomide [59] can lead to the development of secondary leukemia. However, the long-term effect of prolonged exposure to long-term chemotherapy on normal endothelial and vascular tissues is unknown.

6.7 Biomarkers of Clinical Response

Following the preclinical observation that maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial cells (CECs) [60] and that CEC kinetics correlates well with more invasive biomarker of angiogenesis [61, 62], CECs and their progenitor counterpart (CEPs) were measured in the blood of breast cancer patients enrolled in a variety of clinical trials involving metronomic chemotherapy alone or in combination with other drugs. CECs were found to be dynamic markers of clinical response in breast cancer patients receiving CTX and MTX [63], and baseline CECs and CEPs were found to be predictive markers in clinical trials where metronomic chemotherapy was administered along with the anti-VEGF monoclonal antibody bevacizumab [24, 64]. CECs and CEPs have been found to have predictive and dynamic prognostic potential in several other types of cancer in addition to breast cancer, but the wide application of this measurement is still hampered by the lack of simple and standardized procedures [65]. An international effort towards the standardization of CEC and CEP enumeration is currently ongoing. Finally, the study of Dellapasqua et al. [66] has shown that in advanced breast cancer patients, an increase in mean

corpuscular volume of red blood cells may predict response to metronomic capecitabine and cyclophosphamide in combination with bevacizumab. This finding needs to be confirmed in larger clinical trials.

6.8 Conclusion and Future Directions

Metronomic chemotherapy demonstrated activity and provided disease control for patients with metastatic breast cancer. This treatment approach was initially designed to maintain a stable disease as long as possible for metastatic patients that cannot be cured. In fact, the low burden of personal costs for the patient and the possibility to continue the treatment for several months supported the use of metronomic chemotherapy as an additional therapeutic tool for metastatic and pretreated patients with breast cancer. Either elderly patients or those who prefer relatively nontoxic regimens also benefited from this therapeutic option.

However, as results became evident and research for elucidating some conceivably novel mechanisms of action were intriguing, researchers and clinicians started looking for new applications of this therapeutic strategy. On the other hand, not all tumors and especially not all patients derive the same benefit from metronomic chemotherapy. Large and highly aggressive tumors may limit this treatment option, favoring conventional chemotherapy or targeted agents.

Biomarkers are being developed to identify reliable surrogate markers of response and also to identify the proper patients to be treated. Among these, kinetics and viability of circulating endothelial cells (CECs) and progenitor endothelial cells (CEPs) are deemed to be potent predictive tools for patient stratification and treatment monitoring, although the best methods of identification and measurement are still a matter of research.

In fact, it seems unlikely that a single metronomic regimen may have the same efficacy in all patients; the optimal combination regimens, dosing and scheduling of metronomic chemotherapy, remains to be determined for specific breast cancer conditions.

In recent years, the role of metronomic chemotherapy is being studied in the adjuvant setting after a standard adjuvant chemotherapy regimen in the category of ER-negative patients. The CM Maintenance Trial (IBCSG 22-00) investigated a tailored chemotherapy approach for patients with endocrine nonresponsive tumors to reduce the risk of relapse and improve survival. Unlike patients with endocrine responsive disease, who benefit from at least 5 years of endocrine therapy after chemotherapy, patients with endocrine nonresponsive disease do not have the same opportunity.

In the abovementioned trial, 1 year of CM is compared with no further therapy beyond the standard adjuvant program. The trial recently concluded with the enrolment of 1086 patients and the results are eagerly awaited.

As shown in Table 6.2, there are still several ongoing trials to identify the optimal regimen and schedule of metronomic chemotherapy in different settings of patients. Many of them are still investigating specific metronomic regimens in the metastatic

Table 6.2 Ongoing trials with metronomic chemotherapy in breast cancer

Schedule	Phase	Setting	Ref.
Capecitabine 1,000 m/m^2 d1–14 + VNB 60 mg/m^2 d1–8 q21d vs. VNB 50 mg/day d1,3,5/week	II randomized	M+, ≤1 line	NCT01941771
Standard ADJ CT ± capecitabine 650 mg/m^2 bid	III	ADJ, TN	NCT01112826
PTX 100 mg/m^2/week × 8 → DOX 24 mg/m^2/week+ CTX 100 mg/day os × 9 weeks	II	Locally advanced T >2 cm any N TN or inflammatory	NCT01329627
CDDP 25 mg/m^2 d1–3 q3w + CTX 150 mg/day os d1–14	II	M+, TN	NCT01910870
TXT 75 mg/m^2 ± CTX 50 mg/day × 21 day, q3w	II randomized	M+	NCT01526499
Capecitabine 1,500 mg/day + CTX 50 mg/day	II	M+, HER-2 neg	NCT01526512
Capecitabine 1,500 mg/day + AI	II	M+, ER+, postmenopausal	NCT01924078
Pertuzumab + trastuzumab ± CTX 50 mg/day → T-DM1 (if PD)	II randomized	M+, HER-2 pos, ≥60 years	NCT01597414
DOX 24 mg/m^2 + CTX 60 mg/m^2 os/ week × 12 → PTX 80 mg/m^2 + CBDCA AUC 2/ week × 12	II	NeoADJ, TN	NCT00542191
Capecitabine 1,000 mg/day + digoxin	II	M+	NCT01887288
TXT 75 mg/m^2 + capecitabine 1,000 mg/m^2 bid d1–14 × 6 cycles → capecitabine 500 mg/m^2 tid d1–21 vs. 1,000 mg/m^2 bid d1–14	III	M+	NCT01917279
Aflibercept 6 mg/kg iv q3w + capecitabine 1,100–1,600 mg/m^2/day vs. 1,700–2,500 mg/m^2/day × 2 weeks q3w	I	M+	NCT01843725
Bevacizumab 10 mg/kg q2 weeks + PTX 90 mg/m^2 d1,8,15 vs. bevacizumab 10 mg/kg q2 weeks + CTX 50 mg/ day + capecitabine 1,500 mg/day	III	M+, locally advanced	NCT01131195
CM + ASA 325 mg/day (cyc 3–4) × 4 cycles	II	Post-neoADJ (no pCR)	NCT01612247
Capecitabine 700 mg/m^2 bid, d1–14 + etoposide 30 mg/m^2/day, d1–7 q3w	II	M+	NCT01589159
AI + CM + PDN vs. CM + PDN	II	M+, ER+, postmenopausal	NCT00687648
CTX 50 mg/day po + veliparib/placebo	I/II randomized	M+	NCT01351909

Abbreviations: *VNB* vinorelbine, *M* metastatic, *ADJ* adjuvant, *PTX* paclitaxel, *CTX* cyclophosphamide, *DOX* doxorubicin, *TN* triple negative, *CDDP* cisplatin, *CBDCA* carboplatin, *ER* estrogen receptor, *pCR* pathologic complete response, *TXT* Taxotere, *ASA* acetylsalicylic acid, *PDN* prednisone

setting, and most trials are aimed at patients with triple negative disease, because in this setting chemotherapy still represents one of the most reliable option. Nevertheless, there are also trials exploring the role of metronomic chemotherapy in the adjuvant and post-neoadjuvant setting, being this scheduling of chemotherapy possibly useful when target agents are lacking.

A special consideration should be paid to the economic aspects. Given the time being, we cannot avoid to consider the economic impact that the costs of cancer treatments have on public health. In fact, the new targeted treatments often have very high costs. Therefore, therapies such as metronomic CM and similar regimens position themselves as potentially significantly cost-effective palliative treatments for metastatic breast cancer when compared with other novel therapeutic strategies [67].

Finally, the potential development of metronomic chemotherapy in breast cancer cannot disregard the development of research to identify biomarkers and individual tumor characteristics that can better address the use of this treatment strategy in the future.

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Overview of Metronomic Chemotherapy in SWOG Breast Cancer Cooperative Group Clinical Trials

7

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Abstract

Metronomic chemotherapy, defined as continuous or frequent treatment with low doses of anticancer drugs, has been observed to provide excellent safety profiles and has been tested in many tumors. SWOG, formerly the Southwest Oncology Group, has reported extensively on metronomic chemotherapy used in breast cancer. The earliest trials reported on a continuous, or "Cooper-" type, Cyclophosphamide, Methotrexate, 5-Fluorouracil (CMF) regimen in the setting of adjuvant chemotherapy for node-positive breast cancer, in which cyclophosphamide is administered orally on a daily basis and the 5-FU and methotrexate are given by weekly intravenous injection. Subsequently, other regimens have been evaluated. We will, hereby, provide an overview of the main SWOG trials evaluating metronomic chemotherapy in breast cancer.

Metronomic chemotherapy could be defined as "continuous or frequent treatment with low doses of anticancer drugs, often given with other methods of therapy" based on the dictionary of the *Cancer Terms* of the National Cancer Institute. While promising tumor control rates and excellent safety profiles have been observed with metronomic chemotherapy regimens, the selection of patients, drug dosages, and dosing intervals has been somewhat empirical [1]. SWOG, formerly the Southwest Oncology Group, has reported extensively on a continuous, or "Cooper-" type, cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regimen in the setting of adjuvant chemotherapy for node-positive breast cancer, in which cyclophosphamide is administered orally on a daily basis and the 5-FU and methotrexate are given by weekly intravenous injection [2–10]. We will, hereby, provide an overview of the main SWOG trials evaluating metronomic chemotherapy in breast cancer (Table 7.1).

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Table 7.1 Phase III SWOG studies using chemotherapy with a metronomic schedule in breast cancer

SWOG trial	Schedule	Study design	Patients (*N*)	DFS (%)	Median OS (months)	PCR	Reference
S7436	mCMFVP versus L-PAM	Adjuvant	441	48 % versus 35 %, $p = .002$ (at 10 year)	56 % versus 43 %, $p = .005$	NA	Rivkin et al. [4]
S8313	mCMFVP versus FAC-M	Adjuvant	531	NS, 55 % versus 50 %, $p = .06$ (at 5 years)	NS, 64 % versus 61 %, $p = .27$	NA	Budd et al. [10]
S0012	AC+G –T versus 21-day AC-T	Neoadjuvant	372	NS	NS	24.3 % versus 20.7 %, $p = .45$	Ellis et al. [9]
S0221	AC+G vs ddAC –T vs ddP	Adjuvant	3,294	82 % versus 81 %, NS (paclitaxel arms)	–	NA	Budd et al. [11]

DFS disease-free survival, *OS* overall survival, *NS* not statistically significant, *PCR* pathological complete response, *mCMFVP* metronomic cyclophosphamide, methotrexate, fluorouracil, vincristine, prednisone, *L-PAM* single-agent melphalan, *FAC-M* 5-FU 500 mg/m^2 day 1+8, doxorubicin 50 mg/m^2 day 1, cyclophosphamide 500 mg/m^2 day 1, methotrexate 50 mg/m^2 day 22, *AC+G* metronomic cyclophosphamide, weekly doxorubicin 24 mg/m^2, *G-CSF* 21-day AC doxorubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2, *T* paclitaxel 80 mg/m^2/week, *ddAC* dose-dense doxorubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2 IV and pegfilgrastim every 2 weeks, *ddP* dose-dense paclitaxel 175 mg/m^2 every 2 weeks with pegfilgrastim

One of the first clinical trials conducted by SWOG using the metronomic chemotherapy regimen approach for breast cancer, *S7436*, contained continuous CMF and two additional drugs, vincristine and prednisone (CMFVP). Favorable 10-year and 20-year results have been reported with this combination [3, 4]. Four hundred forty-one women with operable breast cancer with histologically positive axillary nodes were randomized to receive either combination cyclophosphamide (60 mg/m^2 orally every day for 1 year), fluorouracil (300 mg/m^2 intravenously [IV] weekly for 1 year), methotrexate (15 mg/m^2 IV weekly for 1 year), vincristine (0.625 mg/m^2 IV for 10 weeks), prednisone (30 mg/m^2 orally days 1–14, 20 mg/m^2 days 15–28, 10 mg/ m^2 days 29–42) (CMFVP), or single-agent melphalan (L-PAM) (5 mg/m^2 orally every day for 5 days every 6 weeks for 2 years) chemotherapy after a modified or radical mastectomy between January 1975 and February 1978 [4]. Patients were stratified according to menopausal status and number of positive nodes (one to three, more than three nodes) before randomization. Maximum duration of follow-up was 12 years, with a median of 9.8 years. The treatment arms were balanced with respect to age, menopausal status, and number of positive nodes. Among eligible patients, disease-free survival and survival were superior with CMFVP ($p = .002$, .005, respectively). At 10 years, 48 % of patients treated with CMFVP remain alive and disease-free, and 56 % remain alive, compared with 35 % alive and disease-free and 43 % alive on the L-PAM arm. Disease-free survival and survival were significantly better with CMFVP compared with L-PAM only in premenopausal patients and patients with four or more positive nodes. Both regimens were well tolerated, although toxicity was more severe and more frequent with CMFVP. This first trial showed that after 10 years of follow-up, adjuvant combination metronomic chemotherapy with CMFVP is superior to single-agent L-PAM in patients with axillary node-positive primary breast cancer. The major advantage was seen in premenopausal women and in patients with more than three positive axillary nodes. Subsequently, a Cancer and Leukemia Group B (CALGB) study, however, failed to identify a benefit to the additional two drugs (VP) in the adjuvant setting [12]. The "classical" CMF became a more widely used combination chemotherapy regimen in breast cancer.

Since the inception of regimens of this type, doxorubicin was developed and found to be one of the most active drugs in breast cancer. Combination chemotherapy regimens that included this drug repeatedly resulted in higher efficacy and improved response rates than regimens that did not [13–15]. A common regimen of this type employed cyclophosphamide, doxorubicin, and 5-fluorouracil (FAC) administered IV every 3 weeks. Subsequently, the National Surgical Adjuvant Breast and Bowel Project (NSABP) reported the results of the NSAPB B-15 trial [15]. This trial compared between "classical" CMF administered for 6 months duration and a short course (12 weeks) of doxorubicin and cyclophosphamide (AC) given IV every 3 weeks for four cycles, with comparable outcomes [14]. This would further suggest increased efficacy for the use of doxorubicin in combination regimens, given the shorter duration of therapy on this arm. This regimen AC (four cycles of doxorubicin in combination with cyclophosphamide IV) became commonly used in breast cancer based on the results of the NSABP B-15 [15].

Subsequently, an intergroup trial led by SWOG, *S8313*, compared 1 year of therapy with "metronomic" continuous CMFVP or 20 weeks of therapy (four 5-week courses) with 5-FU, doxorubicin, cyclophosphamide, and methotrexate (FAC-M) given IV for receptor-negative, node-positive primary breast cancer and showed no difference in overall survival between the two arms, though disease-free survival was marginally superior ($p = 0.06$) on the CMFVP arm [10]. Therefore, FAC-M was not recommended for further investigation or for routine use.

But, since there was a general interest in the FAC regimen, a group of SWOG investigators conducted a pilot toxicity data on a metronomic "weekly continuous FAC" regimen, modeled after SWOG-type CMF, but with the substitution of doxorubicin for methotrexate, in high-risk stages II and III breast cancer patients, administered as adjuvant and neoadjuvant therapy [16]. Neutropenia was dose limiting, so a subsequent study added continuous daily G-CSF overlapping oral cyclophosphamide to overcome this limitation [17, 18]. However, this regimen resulted in significant toxicities including *pneumocystis* pneumonia, thrombocytopenia, and hand-foot syndrome (reaching up to 74 % in patients who received higher chemotherapy doses with G-CSF versus 9 % in patients without G-CSF) [18].

With the relative high incidence of toxicity in the metronomic FAC regimen and the widespread use of the NSABP "AC" combination in the treatment of breast cancer, SWOG next conducted a phase II trial, *S9625*, using weekly doxorubicin with daily oral cyclophosphamide and G-CSF (AC+G) as neoadjuvant treatment of locally advanced breast cancer, after a phase I dose escalation identified the dose of doxorubicin in the 20–22 mg/m^2 week range. *S9625* accrued 122 patients over a 2-year period. Median delivered dose of Adriamycin was 21.8 mg/m^2/week. No treatment related deaths occurred. Dose-limiting toxicity was hematologic: grade 4 neutropenia in 13 patients and grade 3 in 46. No febrile neutropenia was seen. Other grade 4 toxicities included herpetic encephalopathy (1), diarrhea (1), and hematuria (1). In locally advanced breast cancer, the combined rate of pCR (pathologic complete response) including N0 was 21 %. Results appeared especially encouraging for patients with inflammatory breast cancer (IBC; 24 % pCR) and ER-negative disease (pCR 36 %) [19].

Based on these relatively encouraging results of the "metronomic" administration of the AC regimen with continuous G-CSF support, a subsequent SWOG phase III trial, *S0012*, was launched [9]. *S0012* sought to compare the metronomic regimen AC [weekly doxorubicin and daily oral cyclophosphamide with concurrent granulocyte colony-stimulating factor (G-CSF)] over 15 weeks to "standard" AC (doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 intravenously every 3 weeks) for five cycles without growth factor support given before surgery in patients with locally advanced breast cancer (LABC). All patients received paclitaxel given weekly for 12 weeks in view of information that has become available on prolonging adjuvant or neoadjuvant therapy in breast cancer to include the use of a taxane [20–22]. The hypothesis was that the metronomic program would be superior. Three hundred seventy-two patients were randomly assigned to the standard arm ($n = 186$) or the continuous arm ($n = 186$) stratified by disease type (LABC, $n = 256$; IBC, $n = 116$). The primary outcome was pCR at surgery. Secondary outcomes included disease-free survival, overall survival, and toxicity. Results showed that pCR was not different between the

treatment groups stratified by disease type ($p=.42$). In subset analysis, higher pCR rates were observed in the continuous arm versus the standard arm only for stage IIIB disease ($p=.0057$) and in IBC ($p=.06$). More patients in the standard arm had grades 3–4 leukopenia and neutropenia, but there were more instances of stomatitis/pharyngitis and hand-foot skin reaction in the continuous arm. Comparison of overall survival and disease-free survival showed no difference between treatment groups ($p=.37$ and $p=.87$, respectively). The conclusion from *S0112* was that no significant clinical benefit was seen for the metronomic AC+G investigational arm in this trial overall.

A parallel large phase III intergroup SWOG-led trial was being conducted to further test this concept in the adjuvant setting in node-positive and high-risk node-negative operable breast cancer, *S0221*. Eligibility criteria for *S0221* included confirmed diagnosis of operable stage II or III invasive breast cancer, high-risk status based on tumor size or nodal involvement, a history of modified radical mastectomy or local excision of all tumors plus axillary node dissection or sentinel node resection, and no previous history of chemotherapy or radiation. Patients enrolled in the trial were randomized to four treatment arms. Each arm utilized the same three drugs but followed a different treatment schedule in a 2×2 factorial design: dose-dense doxorubicin plus cyclophosphamide every 2 weeks (ddAC) with pegfilgrastim support for 6 cycles or weekly doxorubicin plus daily cyclophosphamide with filgrastim support (AC+G) followed by either 12 cycles of weekly paclitaxel (T) 80 mg/m^2/week or dose-dense paclitaxel (ddP) 175 mg/m^2 every 2 weeks with pegfilgrastim support for 6 cycles. The hypothesis was that the weekly metronomic AC+G regimen is superior to ddAC; and 12 weeks of weekly paclitaxel is superior to ddP ×6. The trial was powered to find a disease-free survival hazard ratio (HR) ≤0.82 for weekly versus 2 week for each factor.

The study closed at a total of 3,294 in January 2012. At the first interim analysis in 2010, the AC randomization was halted for futility. The arms were balanced for standard prognostic factors, and a Cox model adjusting for the paclitaxel arms had a HR = 1.21 (95 % CI 0.98–1.50; $p=0.071$) favoring ddAC. The prescribed boundary for futility was the 99.5 % CI (0.90–1.64) excluding the original alternative hypothesis that HR = 0.82. No boundary was crossed for the paclitaxel comparison, and there was no significant interaction of the two factors. Therefore, the Data Safety and Monitoring Committee recommended stopping randomization to the AC+G arms. Analyses by nodal, hormone-receptor, and HER2 status found no subset in which AC+G appeared superior [23]. S0221 has reopened after amending the protocol such that all patients receive only four cycles of ddAC and are randomized to either weekly paclitaxel ×12 or ddP ×6. Final results were presented at the 2013 American Society of Clinical Oncology (ASCO) meeting [11]. By September 7, 2012, 487 events and 340 deaths had occurred, prompting the third planned interim analysis. The Data Safety and Monitoring Committee recommended reporting the results since the futility boundary was crossed. A Cox model adjusting for the AC arms had a HR = 1.08 (95 % CI 0.90–1.28; $p=0.42$), with the 99.5 % CI excluding the original alternative hypothesis that the HR = 0.82. Estimated 5-year progression-free survivals were 82 % for weekly paclitaxel and 81 % for dose dense q 2-week paclitaxel, not statistically significantly different. Toxicity data were available for 1,385 patients treated with ddP and 1,367 treated with weekly paclitaxel. Grade 5 toxicity occurred in 4 patients on ddP and 2 on weekly paclitaxel. Percent grades

Table 7.2 S0221: comparison of two schedules of paclitaxel as adjuvant therapy for breast cancer [11]

Grades 3–4 toxicity in S0221	Dose-dense paclitaxel (%)	Weekly paclitaxel (%)
Any	36	35
Allergy	14	6
Leukopenia	1	6
Neutropenic fever	<1	<1
Dermatologic	3	0.1
Musculoskeletal pain	11	3
Neurologic	17	10

3–4 toxicity per arm is shown in Table 7.2. Weekly paclitaxel was found to cause more leukopenia but less neurological and musculoskeletal toxicities compared to dose-dense paclitaxel. The overall conclusions from S0221 were that metronomic AC is inferior to dose-dense AC, but either dose-dense paclitaxel every 2 weeks × 6 or weekly paclitaxel × 12 is acceptable schedules of paclitaxel administration.

Most recently, a phase II randomized SWOG trial, S0800, has completed accrual of 214 patients with locally advanced or inflammatory breast cancer randomized to receiving the antiangiogenic agent bevacizumab in combination with chemotherapy (weekly low-dose nab-paclitaxel) followed or preceded by dose-dense AC [24]. The rationale for combining bevacizumab with low-dose "metronomic" weekly taxane was based on reports suggesting that the combination of low, frequent dosage chemotherapy (metronomic chemotherapy) plus an agent that specifically targets the endothelial cell compartment (e.g., TNP-470 and anti-VEGFR2) controlled tumor growth much more effectively than the cytotoxic agent alone [25, 26]. As proposed initially by Klement et al. [25], any anti-vascular effects of the low-dose chemotherapy would be selectively enhanced in cells of newly formed vessels when survival signals mediated by VEGF are blocked. The efficacy of metronomic chemotherapy can be significantly increased when administered in combination with antiangiogenic drugs, such as antibodies against VEGF. *S0800* as designed with randomization between different arms with or without bevacizumab will hopefully provide evidence to test this hypothesis; this would also advance our knowledge and insight about the biology of LABC and IBC as well as the mechanisms at play in anti-VEGF treatment. Results of *S0800* are expected in 2015.

What accounted for the failure of the SWOG continuous metronomic chemotherapy program to improve on the standard?

One possibility is that paclitaxel on a weekly schedule, previously shown to improve outcome over anthracycline-based therapy alone, may be a great leveler, obscuring the effect of scheduling for AC. Second, in the absence of an added drug specifically targeting angiogenesis, any antiangiogenic effects of the continuous approach with chemotherapy may be too weak to be of clinical benefit, or there may be rapid recovery and even overshoot in angiogenesis in remaining tumor after cessation of the therapy, as suggested in some preclinical models [27]. Also, it is possible that there is a deleterious effect that results from the administration of G-CSF in the continuous regimen, due to an upregulation in circulating endothelial progenitor cells, as suggested by Natori et al. [28].

In summary, "metronomic chemotherapy" defined as continuous or frequent treatment of low doses of anticancer agents has been extensively investigated in SWOG trials with favorable results reported in breast cancer (Table 7.1). Single chemotherapeutic agents given in "metronomic" regimens, e.g., cyclophosphamide and taxanes, have proven efficacy and have gained more acceptance than combination chemotherapy such as metronomic AC (weekly doxorubicin plus oral daily cyclophosphamide). The concept of "metronomic chemotherapy" continues to play an important role in the treatment of many cancers including breast cancer. Metronomic chemotherapy would be ideal for the use in patients who have been heavily pretreated with cytotoxic drugs or who have poor performance status or to overcome resistance. However, more appropriate selection of specific drugs and a better definition of "metronomic" doses and schedules for combination chemotherapy are essential to improve tolerability and efficacy. Additional research exploring the combination of metronomic chemotherapy with antiangiogenic drugs is also needed.

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Clinical Trials of Low-Dose Metronomic Chemotherapy in Castration-Resistant Prostate Cancer

8

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Abstract

Low-dose metronomic (LDM) chemotherapy is the continuous or near-continuous use of conventional chemotherapeutic agents at doses that do not necessitate cyclic treatment interruptions. Recently, LDM chemotherapy has gained traction for the treatment of castration-resistant prostate cancer (CRPC). Its excellent safety profile and relatively low rate of severe (i.e., grade 3/4) toxicities make it an enviable treatment, especially for elderly and frail CRPC patients. By searching the MEDLINE, EMBASE, and CENTRAL databases, we identified fifteen published prostate cancer LDM chemotherapy trials comprising 471 patients. The trials were stratified and analyzed according to three common types of LDM regimens: (1) cyclophosphamide monotherapy, (2) cyclophosphamide plus corticosteroid, and (3) complex combination regimens. Oral cyclophosphamide was part of all LDM regimens. Collectively, LDM chemotherapy was found to be beneficial in almost 60 % of patients (mean clinical benefit rate of 58.08 ± 20.30). Severe treatment-associated side effects were rarely seen, with anemia being the most commonly reported. One comparative single-center study showed a superior safety profile and comparable benefit of LDM cyclophosphamide therapy compared to conventional, maximum tolerated dose (MTD) docetaxel

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chemotherapy. Another study highlights that prior LDM chemotherapy does not negatively impact on the subsequent use of MTD docetaxel chemotherapy. In addition, five studies document the benefit of LDM chemotherapy in CRPC patients that have undergone MTD docetaxel chemotherapy. Randomized phase III trials will be needed to allow definitive conclusions as to the clinical utility of the LDM approach in CRPC. Unfortunately, the metronomic use of off-patent drugs such as cyclophosphamide faces unique commercial and regulatory hurdles that are slowing down the clinical development of LDM chemotherapy in prostate cancer and other malignancies.

Abbreviations

bid	Twice a day
CPA	Cyclophosphamide
CRPC	Castration-resistant prostate cancer
LDM	Low dose metronomic (chemotherapy)
MTD	Maximum tolerated dose (chemotherapy)
N/A	Not applicable
od	Once a day
po	Orally
PSA	Prostate-specific antigen
tid	Thrice a day
TTF	Time to treatment failure
TTPP	Time to PSA progression
UFT	Uracil/tegafur

8.1 Introduction

Prostate cancer is the most common non-skin malignancy diagnosed in men. Its incidence and prevalence are peaking in men over 60 years of age, who often also suffer from a number of comorbidities [1]. Despite screening efforts and curative treatment attempts for localized disease, around 25 % of prostate cancer patients present with metastases at diagnosis or during later disease stages. While androgen-deprivation therapy is almost universally applied as the first-line treatment of choice for metastatic prostate cancer, docetaxel chemotherapy is used in only around one-third of patients with castration-resistant prostate cancer (CRPC) [2, 3]. Old age and comorbidities may account for the latter finding. In fact, the use of docetaxel is negatively associated with increasing age of CRPC patients [2]. On the other hand, docetaxel chemotherapy seems to be well tolerated and beneficial in patients up to the age of 80 [4]. Of note, in octogenarians, an individualized treatment approach should be considered.

A recent systematic review of 80 published phase I/II clinical trials studying low-dose metronomic (LDM) chemotherapy documented that this novel form of chemotherapy administration is not only beneficial but that it also excels with an excellent

safety profile [5]. Mean response and median clinical benefit rates were found to be 26.03 % and 46.50 %, respectively. Furthermore, severe side effects were seen in less than 5 % of patients. Aside from breast cancer (26.25 %; $n=21$ studies), prostate cancer was the second most common tumor type studied in LDM chemotherapy trials (11.25 %; $n=9$ studies).

A number of reasons may explain why LDM chemotherapy has gained so much traction in the prostate cancer field. First, the high prevalence of CRPC in elderly and frail patients emphasizes the need for alternative treatment strategies that are more adjusted to this patient population than conventional, maximum tolerated dose (MTD) chemotherapy and its relatively high rate of severe acute toxicities. In fact, safety aspects and quality of life are of paramount importance when it comes to treatment decisions in these patients. In addition, the usually oral and outpatient way of LDM drug administration is particularly appealing to patients with incurable malignancies and limited life expectancy. Second, in the 1980s and early 1990s, cytotoxic treatment of prostate cancer was dominated by metronomic-like oral regimens of cyclophosphamide, etoposide, or estramustine [6, 7]. However, the dosing of such regimens was oriented towards MTD chemotherapy administration, and thus, planned treatment interruptions were common [8]. Third, prostate cancer tumor models have been commonly used in preclinical studies of LDM chemotherapy [9–11]. Fourth, despite recent unprecedented advances in the treatment of CRPC that have seen the approval of potent second-line hormonal therapies, such as abiraterone and enzalutamide, inherent and acquired therapeutic resistance remains a major obstacle to render CRPC a chronic, manageable condition, not to speak of a curable disease [12]. In other words, there continues to be an unmet need for novel treatment modalities. Finally, the economic burden of off-patent drugs such as cyclophosphamide compares favorably to the costs associated with conventional chemotherapy and recently approved targeted anticancer agents [13, 14].

8.2 Overview of Low-Dose Metronomic Chemotherapy Trial Experience in Prostate Cancer to Date

We identified published prostate cancer LDM chemotherapy trials by searching the MEDLINE, EMBASE, and CENTRAL databases (using the keywords "metronomic" and "chemotherapy" and "prostate cancer" or "prostate neoplasm" or "prostate tumour") and by applying the following working definition of LDM chemotherapy, i.e., the continuous or near-continuous use of conventional chemotherapeutic agents at doses that do not necessitate cyclic treatment interruptions to prevent acute treatment-associated toxicities. Although many of the novel targeted anticancer agents are used in a metronomic-like way (i.e., continuously), we focus herein on classical cytotoxic agents as the major component of LDM regimens. We also excluded studies comprising miscellaneous tumor types, including rare cases of prostate cancer [5]. Fifteen studies fulfilled the search criteria and are discussed in more detail.

The study characteristics are summarized in Table 8.1. Briefly, four studies were retrospective chart reviews (27 %) [15–18], nine were prospective,

Table 8.1 Study demographics

	Study design	CRPC stage	Number of patients (evaluable)	Age median (range)	Baseline PSA median (range) ng/mL	Performance status (ECOG)
Lord et al. [19]	Phase II study	Early	80 (58)	69 (51–86)	43.9 (2.4–789)	≤2 (ECOG)
Jellvert et al. [20]	Phase II study	Early	17 (17)	60 (40–75)	N/A	N/A
Vorob'ev et al. [28][a]	Phase II study	Early	25 (25)	72.8 (56–85)[b]	333.1 (±437.4)[c]	≤3 (ECOG)
Nishimura et al. [21]	Phase II study	*Early*-advanced	21 (21)	70 (50–82)	27 (3.6–2,240)	≤3 (ECOG)[d]
Glode et al. [15]	Retrospective chart review	*Early*-advanced	34 (32)	72.6 (54–88)[b]	43.9 (2.4–789)	N/A
Fontana et al. [16]	Retrospective chart review	*Early*-advanced	29 (29)	83 (78–92)	49.4 (6.7–567.8)	≥0 (ECOG)
Hatano et al. [18]	Retrospective chart review	*Early*-advanced	57 (57)	71 (49–90)	27.7 (1.9–7176.0)	≤2 (ECOG)
Nicolini et al. [22]	Phase II study	Early-*advanced*	8 (8)	72 (62–84)	>4–50	≤2 (ECOG)
Fontana et al. [23]	Phase II study	Early-*advanced*	28 (28)	74.5 (54–91)	63.2 (10–5,000)	≤2 (ECOG)
Dickinson et al. [17]	Retrospective chart review	Early-*advanced*	28 (28)	75	123.5 (16–3,448)	N/A
DiLorenzo et al. [29]	Phase I study	Post-docetaxel	16 (16)	67 (46–75)	180 (110–300)	≥1 (ECOG)[d]
Ladoire et al. [24]	Phase II study	Post-docetaxel	23 (23)	74 (55–88)	411 (30–1,760)	≤3 (ECOG)
Nelius et al. [25]	Phase II study	Post-docetaxel	17 (17)	68 (42–85)	134 (11.89–6,554)	≤2 (ECOG)
Gebbia et al. [26]	Phase II study	Post-docetaxel	60 (58)	72 (56–83)	156 (45–478)	≤2 (ECOG)
Meng et al. [27]	Phase II study	Post-docetaxel	28 (28)	72.8 (69–78)	549.5 (50.8–5075.3)	≤2 (ECOG)

Abbreviations: *CRPC* castration-resistant prostate cancer, *PSA* prostate-specific antigen, *ECOG* Eastern Cooperative Oncology Group, *N/A* not applicable

[a]Low-dose metronomic chemotherapy arm

[b]Mean age

[c]Mean (SD)

[d]Karnofsky performance status converted to ECOG performance status

single-arm phase II trials (60 %) [19–27], one was a nonrandomized comparison of CRPC patients undergoing LDM cyclophosphamide monotherapy with a group of patients from the same institution receiving conventional docetaxel chemotherapy (7 %) [28], and one was a prospective phase I trial (7 %) [29]. As is the case with other tumor types, there is no published phase III data available on the use of LDM chemotherapy in prostate cancer but plans for a randomized phase III trial are in motion [30].

All LDM chemotherapy trials accrued patients with CRPC, but the inclusion criteria were often vague, involving patients with (1) early CRPC, patients that had not undergone extensive second-line hormonal therapy attempts, (2) advanced CRPC, patients that had received ≥1 line of second-line hormonal manipulations and/or non-docetaxel chemotherapy, and (3) patients that had undergone docetaxel chemotherapy. Some of the studies comprised patients across these arbitrary categories. Out of the 471 patients enrolled in all these trials, 445 were considered evaluable for response assessment.

Patient age ranged from 40 to 92 years (median age = 72). The average median baseline prostate-specific antigen (PSA) of 146.4 ng/mL is an indication for mostly advanced CRPC stages. With few exceptions [21, 24, 28], the performance status (according to the Eastern Cooperative Oncology Group scale) of study patients was 2 or less. In comparison, contemporary phase III CRPC trials comprise mainly of men with an ECOG performance status of 1 or less, aged 40–95 (median = 69.5), and presenting with an average median baseline PSA (ng/mL) of 103.9 [31–36]. In sum, CRPC patients in the identified LDM chemotherapy trials tend to be older and have higher baseline PSA levels than the prototypical randomized phase III trial CRPC patient. Nonetheless, the patients receiving LDM chemotherapy appear to be largely representative of European or North American CRPC patients, where the majority of the included studies were conducted.

8.3 LDM Chemotherapy Regimens: Clinical Benefit and Side Effects

Table 8.2 depicts the details of the LDM regimens studied. Of note, cyclophosphamide was the chemotherapy backbone of all 15 trials. In 11 trials, cyclophosphamide was flat-dosed at 50 mg po daily. Only four trials studied higher daily cyclophosphamide doses: 50 mg/m^2 od [19], 50 mg po bid [18, 20], or 100 mg po od alternating with 150 mg po od [22]. Fontana et al. also administered a single intravenous bolus of 500 mg/m^2 on the first day of study treatment [23].

8.3.1 Cyclophosphamide Monotherapy

Three clinical trials assessed the activity of cyclophosphamide monotherapy, albeit applying three different cyclophosphamide schedules [19, 22, 28]. Lord et al.

Table 8.2 Treatment regimens

	Treatment					Median duration of treatment [months (range)]
	Chemotherapy drugs			Targeted agents	Corticosteroids	
Lord et al. [19]	CPA 50 mg/m^2 po od					8
Jellvert et al. [20]	Week 1,3,5: CPA 50 mg po bid[b]; ketoconazole 200 mg po tid	Week 2, 4, 6: etoposide 50 mg po bid; EMP 140 mg po bid			Prednisone 10 mg po od	3.5 (0.75–5.5)
Vorob'ev et al. [28][a]	CPA 50 mg po od					6.7 (1–16)
Nishimura et al. [21]	CPA 50 mg po bid	UFT 200 mg po bid	EMP 280 mg po bid			2 (1–3)
Glode et al. [15]	CPA 50 mg po od				Dexamethasone 1 mg po od	9 (95 % CI: 6–14)
Fontana et al. [16]	CPA 50 mg po od			Celecoxib 200 mg po bid	Dexamethasone 1 mg po od	≥3
Hatano et al. [18]	CPA 50 mg po bid	UFT 200 mg bid			Dexamethasone 0.5 mg po bid	4.5 (0.25–45.75)
Nicolini et al. [22]	CPA alternating 100 or 150 mg po od[c]					N/A
Fontana et al. [23]	CPA 50 mg po od[d]			Celecoxib 200 mg po bid	Dexamethasone 1 mg po od	5.4 (1.4–21.5)
Dickinson et al. [17]	CPA 50 mg po od				Dexamethasone 2 mg po od	N/A
DiLorenzo et al. [29]	CPA 50 mg po od			THD 100 or 200 mg po od[e]		N/A
Ladoire et al. [24]	CPA 50 po od				Prednisolone 10 mg po od	N/A
Nelius et al. [25]	CPA 50 mg po od				Dexamethasone 1 mg po od	N/A
Gebbia et al. [26]	CPA 50 mg po od	MTX 2.4 mg po 2×/week				4.5
Meng et al. [27]	CPA 50 mg po od	Capecitabine 1,000 mg po bid		THD 100 mg od	Prednisone 5 mg po bid	6.3 (1.5–20.5)

Abbreviations: *CPA* cyclophosphamide, *UFT* uracil/tegafur, *EMP* estramustine, *THD* thalidomide, *MTX* methotrexate, *od* once a day, *bid* twice a day, *tid* thrice a day, *po* orally, *N/A* not applicable

[a]metronomic arm

[b]one patient received 5 mg od of Idarubicin 4 days/week instead

[c]plus: uromitexan 400 mg po od during 3 of 4 weeks

[d]plus: cyclophosphamide 500 mg/m^2 single iv bolus day 1

[e]plus: Coumadin 1 or 2 mg po od

prescribed cyclophosphamide at 50 mg/m^2, which would correspond to a daily intake of around 90 mg of cyclophosphamide assuming an average body surface area of 1.8 m^2 [37] or even higher given that androgen-deprivation therapy may be associated with significant weight gain in a sizable number of CRPC patients. The daily cyclophosphamide dosing of >50 mg could explain the grade 3/4 lymphopenia rate of 32.8 %. On the other hand, Nicolini et al., who studied a daily alternating oral cyclophosphamide regimen of 100 or 150 mg, reported no instances of lymphopenia. However, grade 2 or 3 neutropenia was noted in all 8 patients, and, as a result, 4 of them came down with infections [22]. In the phase II study by Vorob'ev et al., 50 mg of daily oral cyclophosphamide was not associated with any grade 3 or 4 toxicities at all.

The PSA response rate in the three LDM cyclophosphamide monotherapy studies ranged from 12 to 34.5 % (Table 8.3), with a positive trend for an increasing PSA response rate with an increased daily cyclophosphamide dose. On the other hand, the composite rate of PSA response and PSA stabilization was the highest in the study by Vorob'ev et al. (84 %), and the median response duration was similar in all three trials (around 7.6 months). Inter-study variability and overall small sample sizes, amongst others, preclude definite conclusions about the nature of the association of cyclophosphamide with significant clinical benefit.

8.3.2 Cyclophosphamide plus Corticosteroid Combinations

Cyclophosphamide plus corticosteroid therapy was studied in four trials involving around 25 patients each [15, 17, 24, 25]. Commonly, 50 mg of cyclophosphamide po od was coadministered with varying dosages of dexamethasone (1 or 2 mg po daily) [15, 17, 25] or with 10 mg of prednisolone po od [24]. Since corticosteroids have been shown to be active agents in CRPC, the PSA response and stabilization rates achieved need to be interpreted carefully. They are found to be in the same range as seen with LDM cyclophosphamide monotherapy, as is the case with the median response duration of 6 or 8 months seen in the Glode and Ladoire studies [15, 24].

While the retrospective chart review by Glode et al. did not provide detailed toxicity profiles, Nelius et al. reported no incidence of severe side effects [15, 25]. On the other hand, Ladoire et al. reported severe cases of lymphopenia (26 %), anemia (8 %), and neutropenia (4 %) [24]. Furthermore, the degree of anemia found in 14 % of patients by Dickinson et al. was not specified [17].

8.3.3 Complex Combination Regimens

In attempts to enhance the antiangiogenic effects, LDM cyclophosphamide was combined with the cyclooxygenase-2 inhibitor, celecoxib, in two trials by Fontana et al. [16, 23]. Whereas concurrent cyclooxygenase-2 inhibition appeared to be well tolerated in CRPC patients undergoing LDM cyclophosphamide therapy, the clinical outcome was similar compared to the aforementioned studies of LDM

Table 8.3 Treatment outcome and response

	PSA response (%)	PSA stabilization (%)	PSA progression (%)	Median TTPP [months] (range)	Tumor response rate [RECIST] (%)	Bone metastasis response (%)	Median response duration/TTF [months] (range)	Clinical benefit (%)
Lord et al. [19]	34.5	17.2	48.3	N/A	N/A	N/A	7.5 (CI 3–18)	45
Jellvert et al. [20]	59	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vorob'ev et al. [28][a]	12	72	16	N/A	N/A	N/A	6.3 (2–13)	84
Nishimura et al. [21]	57	24	19	N/A	N/A	10	7 (2–15)	81
Glode et al. [15]	69	6	25	N/A	N/A	N/A	8 (95 % CI 4–10)	75
Fontana et al. [16]	45	17	38	N/A	7	N/A	8.6 (95 % CI 7.6–9.6)	62.5
Hatano et al. [18]	63	26	11	7.2 (4.1–10.1)	29	N/A	N/A	69
Nicolini et al. [22]	25	37.5	37.5	N/A	N/A	N/A	9 (8–31)	62.5
Fontana et al. [23]	32	18	50	3 (2.2–4.2)	17.86	N/A	9.8 (4.9–19.3)	50
Dickinson et al. [17]	46	15	39	4	N/A	N/A	N/A	14
DiLorenzo et al. [29]	15	8	76.9	N/A	N/A	N/A	N/A	23
Ladoire et al. [24]	26	48	22	N/A	N/A	N/A	6 (95 % CI 4–8)	74
Nelius et al. [25]	24	29	47	3 (1–9)	N/A	N/A	N/A	53
Gebbia et al. [26]	25	38	36	N/A	N/A	N/A	4.2 (2–11.2)	63
Meng et al. [27]	35.7	21.4	42.9	2.8 (1–8.6)	N/A	N/A	N/A	57.1

Abbreviations: *PSA* prostate-specific antigen, *TTPP* time to PSA progression, *TTF* time to treatment failure, *RECIST* response evaluation criteria in solid tumors, *N/A* not applicable

[a]Low-dose metronomic chemotherapy arm

cyclophosphamide monotherapy. The longest median response duration of all trials included in our analysis reported by Fontana et al. (9.8 months) might be at least partially attributed to the fact that patients also received a single 500 mg/m^2 bolus cyclophosphamide infusion on the first day of study treatment.

Two studies combined LDM cyclophosphamide with thalidomide, an agent with pleiotropic antitumor effects that also include antivascular activities [27, 29]. In the absence of a trend for increased PSA response rates in both studies when compared to LDM cyclophosphamide monotherapy, the thalidomide-associated toxicities seen by DiLorenzo et al., such as myelosuppression, constipation, neuropathy, and thromboembolic complications, dampen the enthusiasm to further pursue this type of treatment combination. Interestingly, Meng et al. did not report any severe side effects in their patients that also received 5 mg of prednisone po bid and LDM capecitabine.

Four studies explored combinations of ≥2 chemotherapy agents administered concurrently. Nishimura et al. combined cyclophosphamide with the 5-fluorouracil precursor UFT (uracil/tegafur) and with estramustine phosphate [21]. The latter is an estradiol derivative with a nitrogen mustard-carbamate ester moiety with antimicrotubule activities. Likewise, Hatano et al. administered a regimen of cyclophosphamide with UFT and dexamethasone [18]. Gebbia et al. analyzed the benefit of a cyclophosphamide and methotrexate doublet LDM chemotherapy regimen that is commonly used for the treatment of breast cancer [26]. As previously mentioned, Meng et al. combined cyclophosphamide and capecitabine in a regimen also containing thalidomide and prednisone [27]. Despite the limitations of cross-comparing these doublet or triplet LDM chemotherapy regimens with LDM cyclophosphamide monotherapy, the PSA response and stabilization rates were comparable, as were the side effect profiles.

Conventional chemotherapy regimens often comprise several cytotoxic agents with different mechanisms of action that are either scheduled concurrently or sequentially/alternating in an attempt to delay or overcome chemoresistance. Jellvert et al. describe a similar LDM chemotherapy approach based on cyclophosphamide and ketoconazole (an androgen synthesis inhibitor) administration alternating with the use of the topoisomerase II inhibitor etoposide coadministered with estramustine phosphate [20]. Unfortunately, the limited clinical data provided does not allow definitive conclusions as to the clinical potential of this innovative LDM treatment approach. However, the reported incidences of severe toxicities are disconcerting, even though they are based on small absolute numbers given a sample size of 17 patients: thrombocytopenia (24 %), anemia (18 %), heart failure (12 %), abdominal pain (6 %), repeated infections (6 %), pulmonary embolism (6 %), deep vein thrombosis (6 %), acute cholestasis (6 %), weight loss (6 %), and diarrhea (6 %).

8.4 Conventional Versus LDM Chemotherapy

The phase II trial by Vorob'ev et al. is one of the few in the metronomic field that have compared MTD with LDM chemotherapy [28]. Briefly, it is a recent nonrandomized study that compared the efficacy and safety of LDM cyclophosphamide

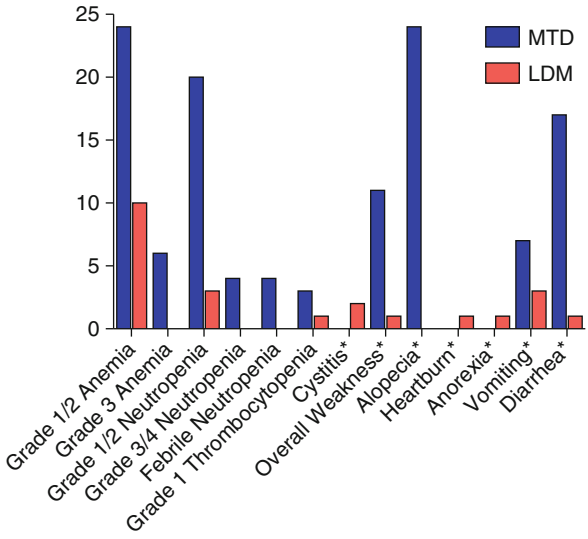

Fig. 8.1 Incidence of adverse side effects in MTD versus LDM treatment arms arising in the study by Vorob'ev et al. [28]: Incidence of adverse side effects, expressed as number of patients affected, between two patient groups treated with either (1) low-dose metronomic chemotherapy (cyclophosphamide) or (2) maximum tolerated dose chemotherapy (docetaxel). *Grade not specified

(50 mg po daily) in 25 early-stage CRPC patients with 30 patients from the same institution that had received MTD docetaxel (75 mg/m^2, every 3 weeks). Vorob'ev et al. did not find significant differences in the median survival time between the LDM and MTD treatment options (15.4 ± 2.2 versus 15.9 ± 1.7 months, respectively). On the other hand, PSA-based parameters, i.e., a PSA response rate of 46.7 % for the MTD arm versus 12 % for LDM cyclophosphamide ($p = 0.02$) with a median PSA stabilization of 6.7 months versus 6.3 months ($p = 0.60$), favored the MTD arm. Likewise, the quality of life assessment using the FACT-P questionnaire (an improvement of 26.7 % in MTD versus 16 % in LDM) and the rate of pain response (according to a visual analogue scale) favored the MTD arm numerically, albeit not statistically significant. While pain reduction occurred in 42.9 % of patients treated with MTD (in contrast to 31.3 % of LDM), the study did not comment on the extent of pain reduction.

With regard to adverse side effects, there is a significant difference between the two treatment options, with consistently lower incidences of side effects occurring in the LDM group (Fig. 8.1). Indeed, no grade 3 or 4 hematologic or non-hematologic toxicities were observed in the LDM group. Overall, Vorob'ev et al. reached the conclusion that, while LDM treatment has some therapeutic significance and is well tolerated, it seemed to be less efficient than standard MTD treatment.

8.5 Low-Dose Metronomic Chemotherapy-Associated Toxicities

Detailed treatment-associated toxicities were reported in 11 LDM chemotherapy trials (Table 8.4). Generally, severe (i.e., grade 3/4) side effects were rarely seen with LDM regimens. This was particularly true for the most commonly used

Table 8.4 Grade 3 and 4 toxicities

	Anemia	Leukopenia	Neutropenia	Thrombocytopenia	Lymphopenia	Hemorrhagic cystitis	Nausea	GI toxicities	Abdominal pain	Acute cholestasis	Peripheral neuropathy	Thromboembolisms	Heart failure	Repeated infections	Weight loss(≥5kg)
Lord et al. [19]	1.7		1.7		32.8										
Jellvert et al. [20]	18			24				6	6	6		12	12	6	6
Vorob'ev et al. [28]															
Nishimura et al. [21]	5	14				5									
Hatano et al. [18]			5												
Fontana et al. [23]															
Dickinson et al. [17]	14														
DiLorenzo et al. [29]	10		37					17			17	17			
Ladoire et al. [24]	8		4		26										
Nelius et al. [25]															
Gebbia et al. [26]		11		3			2								
Meng et al. [27]															

Note: Incidence of grade 3 and 4 side effects (expressed as a percentage of study patients affected)

<5 or not reported
5–10
10–20
>20

cyclophosphamide regimen of 50 mg po daily. Four studies did not report any severe side effects at all [23, 25, 27, 28].

With respect to hematologic toxicities, one needs to consider that cytopenias might also be related to bone-marrow infiltration by prostate cancer cells. On the other hand, it is not unexpected that some of the studies identified patients presenting with severe lymphopenia. In fact, oral cyclophosphamide regimens are used for the treatment of autoimmune disorders, albeit at higher daily doses than 50 mg [38, 39]. Furthermore, total lymphocyte counts may mask the selective depletion of regulatory T lymphocytes by LDM cyclophosphamide, which in turn has been shown to enhance antitumor immunity [40]. While it is not known if immunosuppressive or immunostimulatory cyclophosphamide effects prevail at 50 mg of cyclophosphamide daily, it is reassuring that severe lymphopenias were not accompanied by opportunistic infections [19, 24]. It is similarly reassuring that only 1 out of 471 patients included in the analyses herein developed hemorrhagic cystitis [21].

Compared to LDM cyclophosphamide monotherapy, doublet LDM chemotherapy regimens appear to be similarly well tolerated [21, 26, 27]. However, comedications seem to have contributed to some of the toxicities seen. Specifically, the use of ketoconazole and estramustine likely contributed to the gastrointestinal

and thromboembolic complications reported by Jellvert et al. [20]. In addition, constipation, neuropathy, and thromboembolic events are commonly seen in patients undergoing thalidomide therapy [29]. On the other hand, despite similar thalidomide dosing, no high-grade side effects were seen by Meng et al. [27].

By virtue of its alkylating properties, cyclophosphamide has been shown to increase the risk of secondary malignancies such as leukemia and urothelial cell carcinomas. Dobi et al. recently described a CRPC patient treated with 50 mg of cyclophosphamide daily for 36 months who eventually developed acute myelogenous leukemia characterized by cytogenetic abnormalities frequently observed in alkylating agent-induced leukemias [41]. A cumulative cyclophosphamide dose of >10 g/m^2 (approximate equivalent of 200 days of treatment with 50 mg of cyclophosphamide po daily in a patient with a body surface area of 1.8 m^2) is considered to increase the leukemia risk [42, 43]. While no instances of bladder malignancies were reported, the risk of urothelial cancer doubles for every 10 g cyclophosphamide increment. In addition, treatment duration of more than 1 year was associated with an 8-fold increased risk of bladder cancer [44]. However, no instances of urothelial cell carcinoma were described, admittedly in a patient population with limited life expectancy.

8.6 LDM Chemotherapy for Prostate Cancer: Challenges Ahead

Collectively, LDM chemotherapy was found to be beneficial in almost 60 % of patients (mean clinical benefit rate of 58.08 ± 20.30). In addition, severe treatment-associated side effects were rare. However, there are numerous shortcomings of the evidence published thus far that are worth to be mentioned.

First, our conclusions are based on relatively small and heterogeneous phase I/II trials encompassing 471 patients. While all the trials focused on metastatic CRPC, the extent of pretreatment was highly variable within and between trials. In addition, the study authors used variable endpoint definitions. Second, the term LDM chemotherapy remains vaguely defined. There are no accepted pharmacodynamic surrogate markers to guide proper drug dosing and scheduling. Although cyclophosphamide was the "metronomic backbone" of all LDM regimens, variable cyclophosphamide doses were applied, and cyclophosphamide was combined with a wide array of comedications. Moreover, there is also a lack of detailed knowledge about the benefits of using other drugs than cyclophosphamide in LDM regimens. Third, the limited number of patients studied thus far does not allow definite statements about the rate of rare but potentially clinical significant side effects. Fourth, in the absence of predictive markers of response, all the trials were performed in unselected patients. On the other hand, inherent therapeutic resistance to LDM chemotherapy is common, and acquired resistance develops almost invariably in patients that initially respond to such therapy [11]. While being clearly distinct from resistance to MTD cyclophosphamide [45], the molecular basis of resistance to LDM cyclophosphamide is only poorly understood [11, 46, 47]. In the absence of such molecular information, predictive marker studies performed as part of LDM chemotherapy trials have focused on

angiogenesis-related markers, accounting for the fact that LDM chemotherapy is thought to work primarily via antiangiogenic mechanisms. However, a recent systematic analysis of correlative studies did not reveal consistent results regarding the predictive power of such markers [48]. Interestingly, vascular endothelial growth factor polymorphism analysis of patients of the LDM chemotherapy trial by Fontana et al. discussed herein [23] revealed a highly significant association of the 634CC genotype with treatment outcome [23, 30]. The authors have to be lauded that they plan to validate these findings in a randomized phase III trial.

There are also a number of practical hurdles that slow down the development of LDM chemotherapy towards becoming an accepted treatment modality in prostate cancer. Foremost, there is a lack of phase III trial data. Using cyclophosphamide as an example, it is challenging to obtain industry support for trials with off-patent drugs without commercial interest. On the other hand, studying novel agents combined with LDM chemotherapy is unlikely to result in regulatory approval. Potentially, philanthropic or governmental funding bodies could step in to fill this void. Of note, a pharmacoeconomic evaluation by Bocci et al. suggests that the use of LDM versus MTD chemotherapy may result in cost savings [49]. A "metronomic backbone" may also spare patients the acute side effects typically associated with conventional chemotherapy and may enable the treatment of frail and elderly otherwise not considered for MTD chemotherapy.

It also remains to be seen if there is a role of LDM chemotherapy for earlier stages of prostate cancer. In fact, the beneficial results from randomized phase III trials in early lung and breast cancer applying metronomic-like regimens suggest a role for this treatment modality in the (neo)adjuvant setting, possible also for early prostate cancer [50, 51].

Overseeing the first decade of LDM chemotherapy development in prostate cancer and other malignancies, only history will tell if we are at the end of the beginning or the beginning of the end of rendering this novel use of conventional chemotherapy drugs an accepted treatment modality for prostate cancer. Accounting for the current shift of paradigm towards personalized treatment approaches in cancer therapy, it will be essential to identify pharmacodynamic and predictive markers of response that will provide guidance to use the right chemotherapeutic drug (either alone or in combination) for the right patient with the most suited administration schedule.

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The Role of Metronomic Chemotherapy in the Treatment of Metastatic Colorectal Cancer Patients

9

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Abstract

Neoangiogenesis is a crucial therapeutic target for metastatic colorectal cancer as demonstrated by the effectiveness of biologic drugs with exclusive or partial antiangiogenic activity such as bevacizumab, aflibercept, and regorafenib. Metronomic chemotherapy may be an alternative strategy for targeting tumor angiogenesis and several clinical studies suggested its promising activity and its extremely favorable toxicity profile in the treatment of metastatic colorectal cancer patients.

9.1 Introduction

Tumoral growth, metastatic spread, and disease progression are complex processes to which the formation of new blood vessels contributes significantly [1]. Neoangiogenesis is a crucial therapeutic target for metastatic colorectal cancer (mCRC) as demonstrated by the effectiveness of biologic drugs with exclusive or partial antiangiogenic activity such as bevacizumab, aflibercept, and regorafenib [2–6].

Several studies demonstrated that metronomic chemotherapy may be an alternative strategy for targeting tumor angiogenesis. The continuous administration of low-dose chemotherapy induces microvessel density decrease, hypoxia, endothelial cell alteration and apoptosis, and circulating progenitor cell decrease [7–9].

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Several clinical studies, evaluating metronomic chemotherapy in the treatment of mCRC patients, suggested a promising activity associated with an extremely favorable toxicity profile [10–16].

In the last years, the introduction of new biologic drugs and new treatment strategies has notably prolonged the overall treatment duration of mCRC patients with a subsequent improvement of their outcome. This has required increasing efforts to identify the most convenient approach for limiting as much as possible treatment duration and toxicities. Among the proposed strategies, alternative to the concept of administering consecutive lines of treatment packages until progression or unacceptable toxicity, there is the introduction of maintenance treatment after an induction therapy. In this setting, metronomic chemotherapy, due to its antiangiogenic activity, seems to have a possible rational application: the prevention of progression after an initial tumoral shrinkage at the cost of limited toxicities.

In this chapter, we firstly review the clinical studies evaluating metronomic chemotherapy in the treatment of mCRC patients, and finally we present the MOMA trial, a new perspective in the use and evaluation of metronomic chemotherapy.

9.2 Clinical Studies of Metronomic Chemotherapy in mCRC Patients

Different cytotoxic drugs administered as metronomic regimen in combination with different chemotherapy schedules were evaluated for the treatment of mCRC patients.

One of the first experiences was the phase II study conducted by Lin et al. [12] that analyzed the activity and toxicity of biweekly oxaliplatin plus 1-day infusional 5-fluorouracil/leucovorin (5-FU/LV) followed by metronomic chemotherapy with tegafur/uracil (UFT) in Asiatic mCRC patients resistant to 5-FU. The primary endpoints were response rate (RR) and safety profile, and the study was judged positive if at least 10 responses were observed out of 33 patients. Patients received oxaliplatin 85 mg/m^2 i.v. plus LV 200 mg/m^2 i.v., followed sequentially by 5-FU bolus 400 mg/m^2 and 22-h 5-FU infusion 600 mg/m^2 (25 % of maximum tolerated dose MTD) on day 1, followed by 10 days of daily oral UFT (200 mg/m^2)/LV (30 mg/m^2), with cycles repeated every 2 weeks. The enrollment was prematurely closed after 28 patients due to the achievement of ten responses (RR = 35.7 %). The incidence of grade 3 toxicities (anemia, leukopenia, and vomiting) was less than 5 % with the exception of neurotoxicity (10.7 %). No grade 4 toxicities were observed. Median time to progression and overall survival were 5.2 and 13.4 months, respectively. The authors concluded that oxaliplatin plus infusional 5-FU/LV followed by metronomic UFT was an active regimen with a safe toxicity profile for the treatment of 5-FU-resistant mCRC patients.

Further studies about the role of metronomic chemotherapy in Asiatic mCRC patients were conducted by Ogata et al. A phase II study [13], without a specific statistical design, evaluated the efficacy and the safety of metronomic treatment with weekly low-dosage irinotecan and doxifluridine (5′-DFUR), an intermediate metabolite of capecitabine, in mCRC patients. A total of 45 patients received

irinotecan 40 mg/m^2 (about 25 % of MTD) i.v. on days 1, 8, and 15 every 28 days plus oral 5′-DFUR 800 mg/die on days 3–7, 10–14, 17–21, and 24–28. Thirty patients out of 45 (67 %) did not receive any prior chemotherapy for metastatic disease. The overall RR and disease control rate (DCR) were 35.6 % and 73.3 %, respectively. In the group of patients ($N=30$) who have not been treated for metastatic disease, RR was 40 %, while it was 26.7 % in the group of patients ($N=15$) who received a first-line chemotherapy. The median progression-free survival (PFS) and OS were 187 days (6.2 months) and 452 days (15 months), respectively. Considering safety profile, no grade 4 toxicities were observed, and only 2 % of grade 3 nausea, neutropenia, and diarrhea occurred.

The same group conducted a phase I study [11] to assess the recommended dose of weekly irinotecan combined with oral fluoropyrimidine S-1 as metronomic chemotherapy in 16 mCRC patients who have not received prior treatment for metastatic disease. Patients received a first-line chemotherapy with oral S-1 80 mg/m^2/die on days 3–7, 10–14, and 17–21 plus escalating dose of irinotecan i.v. (starting dose 40 mg/m^2) on days 1, 8, and 15 every 28 days. The results suggested that the recommended dose of weekly irinotecan was 60 mg/m^2.

The activity and safety of the same schedule were evaluated in a phase II study [14]. A total of 45 mCRC patients, untreated for metastatic disease, were enrolled. The primary endpoint was RR. One complete response (CR) and 21 partial responses (PR) were observed, with an overall RR of 48.9 %. At a median follow-up of 21 months, median PFS and OS were 8.1 and 20.9 months, respectively. The observed grade 3–4 toxicities were neutropenia (8.9 %), anemia (4.4 %), anorexia (6.7 %), and diarrhea (6.7 %). On the basis of these results, the combination of metronomic irinotecan and S-1 seems to be a very promising regimen in the first-line treatment of mCRC patients, and it could be due to the combination of cytotoxic activity and inhibition of tumor angiogenesis, as affirmed by authors.

Unlike Asiatic studies, evaluating metronomic chemotherapy also as first-line treatment in mCRC patients, Caucasian studies, presented below, investigated the role of metronomic therapy only in advanced lines of treatment.

One of the first experiences was an exploratory analysis of metronomic irinotecan treatment in chemotherapy-resistant or refractory mCRC patients [15]. The main objective of this study was to evaluate the pharmacokinetic and pharmacodynamic profile of metronomic schedule. Twenty patients received a continuous infusion of irinotecan as follows: irinotecan 1.4 mg/m^2/die ($n=7$), 2.8 mg/m^2/die ($n=5$), and 4.2 mg/m^2/die ($n=8$). Regarding the activity and survival results, 4 patients achieved disease stabilization and the remaining 16 patients progressed at the first evaluation. After a median follow-up of 20 months, median PFS was 2.07 months, and median OS was 8.4 months. No toxicities higher than grade 1 were observed, and no hematological toxicities occurred. The results of this study suggested that metronomic irinotecan in chemotherapy-resistant or refractory mCRC patients could have a potential antitumor effect in the absence of toxicities.

A randomized phase II study [10] evaluated the activity and safety of metronomic cyclophosphamide or megestrol acetate in advanced cancer patients, having exhausted all effective therapies under standard care. The primary endpoint

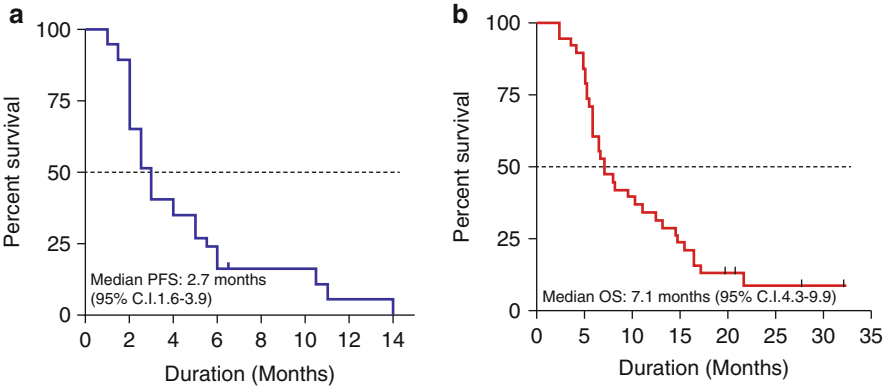

Fig. 9.1 Metronomic tegafur/uracil, cyclophosphamide, and celecoxib schedule in advanced refractory gastrointestinal cancers patients: progression-free survival PFS (**a**) and overall survival OS (**b**) curves

was progression-free rate (PFR) at 2 months, and the treatment was defined effective if at least 5 out of 44 patients (11 %) were free of disease progression at 2 months. A total of 88 patients were randomized (44 patients for arm) to receive oral metronomic cyclophosphamide (50 mg/bid) or megestrol acetate (160 mg/die) until disease progression or intolerance. Twenty-five percent of patients were affected by CRC, while the remaining 75 % by lung cancer, soft tissue sarcoma, melanoma, bladder cancer, gastric cancer, and hepatocarcinoma. Two-months PFR was 9 % in the megestrol acetate arm versus 20 % in the metronomic cyclophosphamide arm. In both arms, no responses were observed, while a stable disease (SD) was observed in 6 patients (5 patients receiving metronomic cyclophosphamide and 1 patient receiving megestrol acetate). One toxic death occurred in the megestrol acetate arm, no grade 4 toxicities were observed, while the incidence of grade 3 toxicities was 4 % in both arms (hormonal and metabolic disorders in the megestrol acetate arm; vomiting in the metronomic cyclophosphamide arm).

This study showed that only metronomic cyclophosphamide seems to be active and safe in the treatment of pretreated patients with advanced solid cancer.

A further phase II study [16] evaluated metronomic cyclophosphamide in association with metronomic UFT plus celecoxib in heavily pretreated patients with advanced gastrointestinal cancers. A total of 38 patients were enrolled, and among them 30 (79 %) had a diagnosis of mCRC. Patients received cyclophosphamide 500 mg/m^2 i.v. on day 1 and from day 2 oral cyclophosphamide 50 mg/die plus UFT 100 mg/bid and celecoxib 200 mg/bid until disease progression or intolerance. The primary endpoint was PFR at 2 months, and the study treatment was considered promising if at least 11 patients were progression-free at 2 months. The study met its primary endpoint: 17 patients were progression-free at 2 months with disease stabilization. No responses were observed. After a median follow-up of 18.3 months, median PFS and OS were 2.7

Table 9.1 Clinical studies evaluating metronomic chemotherapy in mCRC patients

Study	Phase	Line	Tumor	Regimen	Patients (*n*)	RR (%)	PFS (mos)	OS (mos)	Toxicity G3/G4 (%)
Lin et al. [12]	II	Advanced line	mCRC	FOLFOX i.v. d 1 → orally UFT/LV d 1–10; every 14 days	28	35.7	5.2	13.4	<5
Ogata et al. [13]	II	I or II line	mCRC	CPT-11 i.v. d 1, 8, 15+ orally 5-DFUT d 1–28; every 28 days	45	35.6	6.2	15	2
Ogata et al. [14]	II	I line	mCRC	CPT-11 i.v. d 1, 8, 15 + orally S-1 d 3–7, 10–14, 17–21, every 28 days	45	48.9	8.1	20.9	<10
Allegrini et al. [15]	NA	Advanced line	mCRC	CPT-11 i.v. continuously	20	0	2.07	8.4	0
Allegrini et al. [16]	II	Advanced line	GI (79 % mCRC)	CTX i.v. d 1 → orally CTX + UFT + CXB, from d 2 continuously	30	0	5.1	12.1	0
Marmorino et al. [17]	II	Advanced line	mCRC	Orally Cape + CTX continuously	26	NA	2.1	6.0	0

UFT tegafur/uracil, *LV* leucovorin, *CPT-11* irinotecan, *5-DFUT* doxifluridine, *S-1* tegafur-gimeracil-oteracil, *CTX* cyclophosphamide, *CXB* celecoxib, *Cape* capecitabine, *i.v.* intravenous, *mos* months, *d* day, *mCRC* metastatic colorectal cancer, *GI* gastrointestinal cancer, *n* number, *NA* not available, *G* grade

and 7.1 months, respectively (Fig. 9.1). Considering safety profile, metronomic cyclophosphamide plus UFT and celecoxib resulted as a very safe regimen with only grade 1 toxicities.

These promising results were prospectively evaluated by a recent phase II study [17] that investigated the activity of metronomic capecitabine and oral cyclophosphamide in refractory mCRC patients. Twenty-six patients received capecitabine 800 mg/bid and oral cyclophosphamide 50 mg/die until disease progression or unacceptable toxicity. The primary endpoint was PFR at 2 months, and the study treatment was considered promising if at least six patients were progression-free at 2 months. The study did not reach its primary endpoint: only five patients were progression-free at 2 months. At a median follow-up of 14.4 months, median PFS and OS were 2.1 and 6.0 months, respectively. Metronomic capecitabine and cyclophosphamide showed a low clinical activity in heavily pretreated mCRC patients.

On the basis of the results presented above, we can conclude that the role of metronomic chemotherapy in heavily pretreated mCRC patients is still not well defined, while it seems to be clearer in the first line of treatment, although the studies evaluated only Asiatic patients.

The main clinical studies, reported in this paragraph, are summarized in Table 9.1.

9.3 The MOMA Trial: A New Perspective

The MOMA trial [18] is a prospective, open-label, multicenter randomized phase II study, conducted by the Italian GONO Group (Gruppo Oncologico Nord Ovest), with the objective to evaluate maintenance treatment with bevacizumab alone or bevacizumab plus metronomic chemotherapy, with capecitabine and oral cyclophosphamide, after a 4-month first-line induction treatment with FOLFOXIRI plus bevacizumab in mCRC patients. The rational of this study is based on the following data: (1) the induction treatment with FOLFOXIRI plus bevacizumab followed by maintenance with bevacizumab +/– 5-FU is an efficacious option for first-line treatment of mCRC patients [19, 20]; (2) CAIRO-3 trial demonstrated that maintenance with standard doses of capecitabine plus bevacizumab provides a significant 2nd progression advantage, as compared to observation alone, although at the cost of some toxicities that limited the rate of reintroduction of CAPOX [21]; (3) metronomic chemotherapy may represent an alternative and better-tolerated strategy for targeting tumor angiogenesis, and preclinical evidences show that it may synergize with bevacizumab in order to maximize the anti-angiogenic effect [9, 22, 23]. The combination of metronomic capecitabine and cyclophosphamide with bevacizumab was evaluated in advanced breast cancer patients, and it showed a promising activity (RR=48 %) with a good safety profile [24].

In the MOMA trial, patients with unresectable and measurable mCRC, not previously treated for metastatic disease, are randomized (1:1) to receive 8 cycles of induction chemotherapy with FOLFOXIRI plus bevacizumab (5-FU 3,200 mg/m^2 i.v. continuous infusion day 1; LV 200 mg/m^2 i.v. day 1; oxaliplatin 85 mg/m^2 i.v. day 1; irinotecan 165 mg/m^2 i.v. day 1; bevacizumab 5 mg/kg i.v. day 1; every 2 weeks), followed by maintenance treatment with bevacizumab alone (bevacizumab 7.5 mg/kg i.v. day 1 every 3 weeks) or bevacizumab (same schedule) plus metronomic oral capecitabine 500 mg/tid and oral cyclophosphamide 50 mg/die continuously. Both regimens are administered until evidence of disease progression or intolerable toxicity (Fig. 9.2). Patients that progress during maintenance therapy can be retreated with FOLFOXIRI plus bevacizumab or with a modified FOLFOXIRI plus bevacizumab regimen (i.e., dose reductions or single drug interruptions) for four cycles as a rechallenge, followed by maintenance with bevacizumab alone or in association with metronomic chemotherapy (according to randomization arm).

The primary endpoint is PFS, and it is measured from the day of randomization until the first observation of disease progression or death due to any cause. Disease evaluation is performed every 8 weeks with a computed tomography scan of the chest and abdomen. The secondary endpoints are RR, OS, safety profile, resection rate, duration of response, time to strategy failure, time to second disease progression, and the evaluation of potential surrogate markers of bevacizumab and metronomic efficacy (including pharmacokinetic, pharmacodynamic, and pharmacogenetic parameters).

According to the statistical design suggested by Rubinstein and Korn, estimating a first-line PFS with chemotherapy plus bevacizumab of 11 months, to detect an HR of 0.75 in favor of the experimental arm, with a power of 80 % and a type-I error (one sided) of 15 %, a total of 173 events are required. Considering an enrollment rate of 90 patients/year and a minimum period of follow-up of 18.0 months, a total of 222 patients should be randomized (111 per arm).

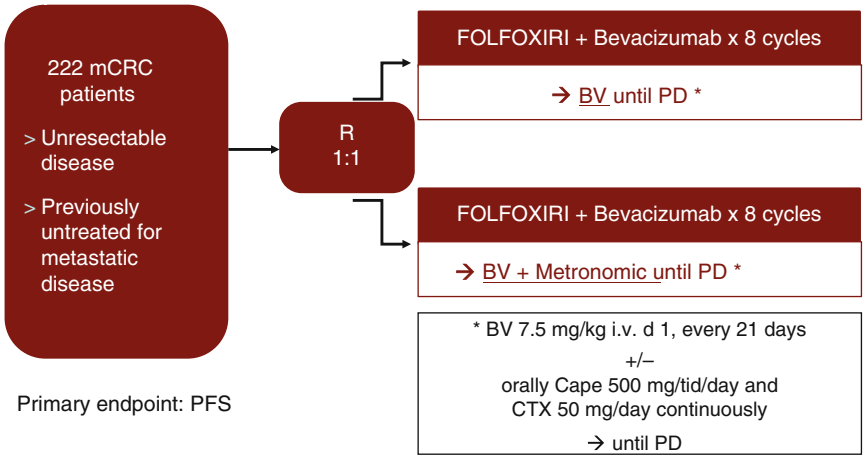

Fig. 9.2 MOMA study design. *mCRC* metastatic colorectal cancer, *PFS* progression-free survival, *PD* progression disease, *BV* bevacizumab, *Cape* capecitabine, *CTX* cyclophosphamide, *i.v.* intravenous, *tid* thrice, *d* day, *beva and metronomic schedule

From May 2012 to February 2014, 126 patients have been so far enrolled in 14 Italian centers, and the study is still recruiting.

The MOMA trial is the first randomized study comparing two different maintenance schedules: bevacizumab alone (as usually used in clinical practice) versus bevacizumab plus metronomic capecitabine and cyclophosphamide after an intensive 4-month induction therapy in mCRC patients. The results of the MOMA trial will suggest if metronomic chemotherapy can really maximize the antiangiogenic effect of bevacizumab and if this combination, after a short induction treatment with full-dose chemotherapy plus bevacizumab, may be an effective approach for prolonging disease control in mCRC patients.

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Clinical Activity of Metronomic Chemotherapy in Central Nervous System Cancers

10

Doo-Sik Kong and Do-Hyun Nam

Abstract

Despite recent advances glioblastoma (GBM), which is the most frequent malignant central nervous system (CNS) tumor, remains a lethal disease. One of characteristics in malignant gliomas is the robust and aberrant vasculature within the tumor which could be the target for the frequent administration of low-dose metronomic chemotherapy. This chapter will mainly focus on determining current status and recent trends of metronomic treatment for CNS tumor by review of published literatures. Indeed, many preclinical evidences support the metronomic use of camptothecins and temozolomide in glioblastoma models and numerous clinical applications of metronomic regimens (e.g. etoposide, temozolomide) for adult patients with tumors of CNS have been described. Moreover, metronomic treatment may serve as a useful platform for combination strategies in certain CNS tumors.

Despite recent advances such as improved resolution of diagnostic imaging, innovating navigation-guided surgical technique, and continuous introduction of new chemotherapeutic agents, glioblastoma (GBM), which is the most frequent malignant central nervous system (CNS) tumor, remains an essentially lethal disease. The gold standard treatment modality for GBM has been known as concurrent chemoradiotherapy using temozolomide (TMZ) (75 mg/m^2/day for 6 weeks) followed by cyclic TMZ chemotherapy (150 or 200 mg/m^2 for 5 days per 28-day cycle; 5/28 dosing schedule) [1]. Nevertheless, their prognosis is still extremely poor and most of tumors recur within 2 years from the first diagnosis. In addition, there is no consensus about the optimal regimen for recurrent GBM.

One of the characteristics of malignant gliomas is robust and aberrant vasculature within the tumor. Thus, tumor-targeting therapies may be promising; however,

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conventional administration of chemotherapeutic agents requires a treatment-free period for the recovery of normal host cell, which induces tumor endothelial cells (EC), and may have enough chance to repair the damage caused by the chemotherapy and resumes tumor regrowth during this treatment-free interval [2]. Tumor EC have been thought as optimal candidate for therapy targeting those EC [3–5].

Since Browder and colleagues [2] and Klement and colleagues [5] published groundbreaking preclinical studies in 2000, frequent administration of low-dose metronomic chemotherapy could produce potent anticancer effects through the inhibition of angiogenesis. To overcome these limitations of conventional chemotherapeutic regimen requiring treatment-free period and to target vascular EC of tumor, frequent systemic administration or continuous infusion with cyclophosphamide at a minimally toxic dose inhibited basic fibroblast growth factor (bFGF)-mediated angiogenesis and induced the apoptosis of vascular ECs in tumor microvessel. This frequent chronic dosing of cyclophosphamide targeted the tumor endothelium, resulting in endothelial apoptosis followed by tumor cell death [2].

This paper will mainly focus on determining current status and recent trends of metronomic treatment for CNS tumor by review of published literatures.

10.1 Postulated Mechanism of Metronomic Treatment for CNS Tumor

Metronomic treatment may be feasible for CNS tumor, as it is still effective in other cancers from each organ. The effect of metronomic treatment for CNS tumor can be explained by the two following mechanisms.

10.1.1 Antiangiogenic Mechanism

GBM has highly proliferative vasculature within the tumor. To effectively approach CNS tumor, there is one limitation that chemotherapeutic agent should be permeable to the blood–brain barrier (BBB) in the CNS. In terms of permeability of BBB, targeting the vasculature must be very attractive in the field of CNS tumor. Treatment strategy without need to penetrate beyond the blood–brain barrier makes them particularly suited to neuro-oncology [6, 7]. Metronomic chemotherapy has a direct toxic effect on EC in tumor vasculature and is postulated to have combined antitumor and antiangiogenic effects.

10.1.2 Consumption Mechanism of Repair Enzyme

TMZ is one of the most effective chemotherapeutic agents against malignant glioma and acts by methylating bases within DNA, which subsequently produces DNA double-strand breaks and induces apoptosis [8]. The DNA damage inflicted by TMZ is

repaired by the cellular repair enzyme O6-methylguanine-DNA methyltransferase (MGMT). The predominant pathway for the repair of O(6)-methylguanine in DNA is via the activity of a methyltransferase protein that transfers the methyl or alkyl group to a cysteine acceptor site on the protein itself [9]. Resistance to TMZ or BCNU is known to be mediated by expression of the MGMT repair enzyme [10, 11]. Because MGMT enzyme is irreversibly inactivated during DNA repair process, the enzyme needs to be continuously refueled by de novo protein synthesis. Therefore, continuous use of TMZ may lead to MGMT depletion by consumption and results in overcoming the inherent resistance of glioma cells [8]. It may improve the efficacy of TMZ, particularly in patients with tumor harboring unmethylated MGMT promoter gene.

10.2 Preclinical Evidences for CNS Tumor

Based upon pioneering research works in various kinds of tumor model [2, 5], Bello and colleagues [12] showed that metronomic etoposide plus antiangiogenic therapy prolonged survival in orthotopic, intracranial U87 GBM xenografts, compared with conventionally dosed chemotherapy with or without antiangiogenic therapy. Takano and coworkers [13] reported that irinotecan (CPT-11) had a direct antiangiogenic effect on endothelial cells and indirectly on glioma cells via downregulation of hypoxia inducible factor-1α (HIF1α) and vascular endothelial growth factor (VEGF). Many antitumor chemotherapeutic agents, including CPT-11, have been tested clinically for their antiangiogenic potential in systemic cancer [14]. The antiangiogenic properties of the camptothecins: 9-AC, topotecan, gimatecan, and CPT-11, have been demonstrated in both in vitro and in vivo assays [2, 5, 15].

Kurzen and colleagues [16] demonstrated that angiogenesis was significantly inhibited by 5 microM TMZ in the chorioallantoic membrane assay, which also found to be effective in interfering with in vitro angiogenesis as measured by the Matrigel assay.

Kim and colleague [17] investigated the antitumor efficacy of metronomic administration of low-dose TMZ in in vitro cell proliferation/cytotoxicity assay and in vivo rat and nude mouse orthotopic glioma model. They found that frequent administration of TMZ markedly inhibited angiogenesis as well as tumor growth in a TMZ-resistant C6/LacZ rat glioma model. For the TMZ-sensitive U-87MG cells, even with a very low dose of TMZ, which was not effective to reduce tumor mass, the metronomic treatment of TMZ reduced the microvessel density in a nude mouse orthotopic model.

10.3 Clinical Applications for CNS Tumor

10.3.1 Recurrent Glioblastoma

In the beginning, etoposide (VP-16) had been regarded as optimal candidate for low-dose metronomic treatment for recurrent glioma [18, 19]. As scientific evidences of the efficacy of low-dose metronomic treatment as antiangiogenic action

are accumulating [2, 5, 7, 12, 14, 16, 20], many authors hypothesized that metronomic schedule of etoposide would enhance the antitumor activity of combined chemotherapeutic agents based on potentially complimentary mechanisms of antiangiogenic action.

Kesari and colleagues [21] performed phase II study of metronomic schedule of low-dose etoposide (35 mg/m^2 daily for 21 days), alternating every 21 days with cyclophosphamide (2 mg/kg daily for 21 days), in combination with daily thalidomide and celecoxib, in adult patients with recurrent malignant gliomas. However, in 28 recurrent GBMs, outcome of metronomic treatment was disappointing (6-month progression-free survival (6PFS) was only 9 %). The authors suggested that further metronomic chemotherapy combined with more potent antiangiogenic agents for less advanced tumors might be warranted. In 2009, Reardon and colleagues [22] reported clinical outcome of metronomic treatment with new combination of daily oral etoposide (50 mg/m^2 for 21 days) and bevacizumab (10 mg/kg biweekly) for a total of 59 recurrent malignant gliomas (27 GBM and 32 grade III anaplastic glioma). In their study, 6PFS of grade III and GBM was 40.6 and 44.4 %, respectively. They also demonstrated that hypertension predicted better outcome, whereas high carbonic anhydrase 9 and low VEGF were associated with poorer progression-free survival.

Since additional preclinical studies regarding metronomic chemotherapy with TMZ had been introduced [16, 17, 23], metronomic TMZ has emerged, as a well-tolerated salvage approach in recurrent GBMs. TMZ has many reasons of becoming a good alternative for metronomic treatment instead of etoposide (Table 10.1). First, TMZ is an alkylating chemotherapeutic agent that is the gold standard of care for newly diagnosed GBM. Low-dose continuous TMZ can have antiangiogenic action as well as potent alkylating action when used at maximal tolerated dosage (MTD). If chemoresistance of the tumor to MTD of TMZ is expected during concurrent chemoradiotherapy or cyclic TMZ chemotherapy period, low dose of TMZ can be rechallenged as metronomic treatment strategy without requiring new drug in this heavily pretreated population. This concept of chemo-switch schedule is one of the potent advantages of metronomic TMZ. Second, TMZ is an oral agent and well tolerated; as a result, it provides better compliance. Third, it has modest toxicities, of which most are grade I or II event and is very rare to discontinue treatment protocol. In addition, it can provide a relatively good performance status in terms of health-related quality of life (QOL), leading to preserved QOL in GBM patients

Table 10.1 Temozolomide-based metronomic chemotherapy

Advantage	Weakness
Good tolerance	Ill-defined treatment failure with pseudoprogression
Appropriate and well-designed clinical trials	Absence of biomarker
Preclinical evidence	Unknown genetic background
Low toxicity rate	
Concept of chemo-switch schedule	
Good performance status	
Inhibition of MGMT activity	

[24]. Finally, it is likely that the combination of metronomic chemotherapy with more potent inhibitors of angiogenesis or more effective agents inhibiting invasion will lead to greater antitumor activity.

Based upon those benefits of TMZ as metronomic treatment, Brandes and colleagues [25] modified dosage of TMZ as 75 mg/m^2/day for 21 days every 28 days and showed good results of 30.3 % 6PFS in chemonaive GBM patients with recurrence or progression.

Perry and colleagues [26] rechallenged with continuous dose-intense TMZ 50 mg/m^2/day for recurrent GBM patients who underwent standard TMZ 150–200 mg/m^2×5 days in a 28-day cycle for three or more cycles. In particular, they stratified patients with 91 recurrent GBM into three groups according to prior duration of treatment with TMZ and time of progression: group B1 (early), GBM with progression while receiving adjuvant TMZ before completion of six cycles of adjuvant TMZ; group B2 (extended), GBM with progression while receiving extended adjuvant TMZ beyond the standard six cycles but before completion of adjuvant treatment; group B3 (rechallenge), GBM with progression after completion of adjuvant treatment and a treatment-free interval of greater than 2 months. As a result, the extended group did not seem to benefit (6PFS: 7.4 %), but the early and rechallenge groups achieved 6PFS of 27.3 and 35.7 %, respectively. It can be interpreted that one of the groups could have harbored tumors with a different biology, considering that the responsive rate to metronomic TMZ had difference based on the timing of failure [27].

Kong and colleagues [24, 28] performed a pilot study and subsequent phase II trial of metronomic treatment for recurrent GBM. They showed that 6PFS was 32.5 % and the 6-month overall survival (OS) was 56.0 % in all 38 patients. In addition, they assessed quality of life with Short Form-36 between pre- and post-metronomic treatment and showed that the quality of life was relatively well preserved even after metronomic treatment.

Recently, Omuro and colleagues [27] performed similar metronomic TMZ schedule (50 mg/m^2/day) for 37 recurrent GBMs. They showed that 6PFS was 19 % and 1-year OS was 35 %. In particular, they analyzed the impact of prior bevacizumab, which is a potent antiangiogenic chemotherapeutic agent, on outcome and demonstrated that patients with bevacizumab failure survived significantly less than bevacizumab-naive patients (28 % vs. 84 %). These metronomic studies of TMZ [24, 27] represented that MGMT promoter methylation status was not associated with the outcome of metronomic treatment, although analyses were limited by the small number of patients in whom this could be determined accurately. Stockhammer and coworkers [29] reported the efficacy of metronomic low-dose temozolomide in combination with celecoxib that 6PFS was 43 % in 28 patients with recurrent malignant gliomas.

10.3.1.1 Overcome Resistance of MGMT

Tolcher and colleagues [23] demonstrated that a protracted schedule of TMZ markedly inactivated the activity of MGMT. They measured O(6)-alkylguanine-DNA alkyltransferase activity in the peripheral blood mononuclear cells of patients treated on

two phase I protracted temozolomide studies. Temozolomide plasma levels on MGMT inactivation and regeneration, as well as the relation between MGMT inactivation and toxicity, were studied. As a result, they observed that marked inactivation of MGMT occurred following 7, 14, and 21 days of temozolomide treatment, with mean MGMT activity decreasing by 72 %. They showed that protracted schedules could lead to an "autoenhancement" of temozolomide's inherent cytotoxic potential by cumulative reduction of the cell's capacity for MGMT-mediated DNA repair and resistance.

To date, many authors reported that MGMT methylation status had no prognostic impact in the setting of low-dose metronomic TMZ treatment for recurrent malignant gliomas [24, 26, 29]. According to the studies by Perry and colleagues and Kong and colleagues [24, 26], they assessed MGMT promoter methylation status when the initial surgical resection or biopsy specimen was obtained. It had some possibilities that selection of methylated tumor or induction of MGMT by previous chemoradiotherapy could be ruled out. Considering these drawbacks, Stockhammer and colleagues [29] investigated the impact of MGMT methylation status when assessed immediately before start of metronomic treatment.

However, the hypothesis that the schedule-dependent antitumor activity of TMZ is a result of cumulative depletion of MGMT and treatment efficacy may increase with more protracted TMZ dosing remains controversial. In randomized trial studied by Brada and colleagues [30], they compared TMZ schedules and demonstrated that a 21-day schedule (100 mg/m^2 for 21 days, 111 patients) was inferior to the 5-day schedule (200 mg/m^2 for 5 days, 112 patients) for recurrent high-grade gliomas and also showed a 2-month decrease in median survival ($P = 0.056$). In this study, the authors suggested that peak TMZ concentrations, rather than prolonged exposure, may be most important for treatment efficacy. They also suggested that reduced TMZ concentrations might also lead to lower concentration available in the CNS as a result of reduced brain penetration.

10.3.1.2 Limitation of Interpretation

To date, metronomic treatment with TMZ alone or combined with chemotherapeutic agents showed good clinical outcome. Recent review by Lien and colleagues [31] showed that phase II evidence comprising of almost 3,700 patients indicates that low-dose metronomic chemotherapy is active and can be safely administered.

Nevertheless, some issues need to be addressed. One important pitfall in interpreting treatment of recurrent glioblastoma is the possible treatment of pseudoprogression [29]. Most frequently within 3 months, up to 13.7 % of patients treated with combined chemoradiotherapy reveal progressive contrast enhancement without histological evidence of tumor recurrence [32]. In the recurrent GBM, it is very difficult to interpret treatment failure or pseudoprogression, when the tumor shows newly enhanced pattern or enlarged mass on the magnetic resonance imaging [33, 34]. Currently, the validity of progression-free survival as the primary end point in many clinical trials and eligibility in salvage treatment trials have been limited because of erroneous interpretation of the pseudoprogression. In addition, non-successive nonrandomized phase II trials must be exposed to inherent selection bias [35].

10.3.2 Newly Diagnosed GBM

To date, there has been little information about the effect of metronomic treatment for newly diagnosed GBM. Tuettenberg and colleagues [36] demonstrated that metronomic temozolomide in combination with the COX-2 inhibitor rofecoxib was feasible, safe, and maintained a good quality of life for 13 patients with glioblastoma, especially those with tumors characterized by high angiogenic activity. For the whole study population, median time to progression and overall survival were 8 months and 16 months, respectively. Immunohistochemistry suggested that tumors with higher vessel densities were characterized by a significantly better control than those with lower vessel densities.

Clarke and colleagues [37] performed randomized phase II study for 43 patients with newly diagnosed GBM. They administered daily TMZ 50 mg/m^2 and showed that median overall survival (OS) and PFS were 15.1 months (95 % CI, 12.3–18.9 months) and 5.0 months (95 % CI, 4–6.7 months). Their study did not show evidence that metronomic temozolomide was superior to the standard dosing, although this schedule was well tolerated.

In the recent phase III study for newly diagnosed GBM by Gilbert and colleagues [38], no statistically significant difference was observed between arms for median OS (16.6 vs. 14.9 months, respectively; hazard ratio [HR], 1.03; $P = 0.63$) or median PFS (5.5 vs. 6.7 months; HR, 0.87; $P = 0.06$), comparing dose-dense TMZ schedule (5–100 mg/m^2 days 1–21 every 28 days for 12 cycles maximum with standard TMZ schedule) with a standard TMZ schedule (150–200 mg/m^2 days 1–5 every 28 days for 12 cycles maximum). This implies that metronomic treatment has still limitation to deplete cellular MGMT and restore sensitivity to temozolomide.

10.3.3 Pediatric Brain Cancer

In the field of pediatric brain cancer, metronomic treatment has been tried earlier. In 1997, Needle and colleagues [39] performed phase II study of daily oral etoposide (50 mg/m^2/day for 21 consecutive days every 28 days) for pediatric recurrent brain tumors. Three of four patients with PNET (primitive neuroectodermal tumor)/medulloblastoma achieved a PR and two of five with ependymoma responded, one with a CR and one with a PR.

The pediatric oncology group performed large series of metronomic treatment for 78 pediatric brain tumors [40]. Eight doses of methotrexate (MTX) 7.5 mg/m^2 every 6 h were administered on a weekly schedule for as long as 18 months. They concluded that low-dose oral MTX showed no significant activity against malignant glioma, medulloblastoma, brainstem tumors, and miscellaneous histologic types.

Baruchel and colleagues [41] investigated the effect of TMZ metronomic treatment for 28 recurrent pediatric brain tumors in 2006. Because disease criteria were heterogeneous, it was difficult to interpret the efficacy of metronomic

treatment. Four patients (15 %) had objective response rate (2 CR and 2 PR) during the treatment period. They also stratified patients into heavily treated and non-heavily treated group; however, they did not find any difference between them. This study suggested the feasibility of metronomic treatment for pediatric brain tumors.

Peyrl and colleagues [42] reported antiangiogenic multidrug combination metronomic therapy using bevacizumab, thalidomide, celecoxib, fenofibrate, etoposide, and cyclophosphamide in 16 patients with recurrent embryonal brain tumors. Seven patients with medulloblastoma had 100 % 6PFS, while four patients with CNS primitive neuroectodermal tumors showed 0.00 % 6PFS. Their study suggested that antiangiogenic metronomic treatment is particularly beneficial for patients with medulloblastoma.

Recently, phase II study by Robison and colleagues [43] may be one of large series of metronomic treatment in the field of pediatric brain cancer. They performed a prospective, open-label, single-arm, multi-institutional phase II study to evaluate the efficacy of a "5-drug" oral regimen (21-day cycles of low-dose oral cyclophosphamide and etoposide, with continuous oral thalidomide, celecoxib, and fenofibrate) in children with recurrent or progressive cancer. A total of 97 patients were treated in this study from 2005 to 2009, and 69 heterogeneous pediatric brain tumors were included in this study. Clinical outcome of 5-regimen metronomic trial was heterogeneous: high-grade glioma, 1 (5 %); ependymoma, 7 (37 %); low-grade glioma, 7 (58 %); and medulloblastoma/PNET, 1 (39 %) successfully completed in 27 weeks of the 5-drug regimen without PD or significant toxicity.

For newly diagnosed brainstem glioma, Sharp and colleagues [44] performed standard radiotherapy and concomitant metronomic TMZ at 85 mg/m^2/day for 6 weeks, followed by metronomic TMZ monotherapy at the same dose. In 15 patients enrolled, median time to progression was 5.13 months (95 % CI = 6.4, 10.8), and median overall survival (OS) was 9.8 months (95 % CI = 6.4, 10.8). They concluded that there was no added benefit for patients compared to radiotherapy alone, despite promising results of previously published studies of metronomic dosing.

Conclusion

Based upon the published literature, clinical outcome of metronomic treatment for CNS tumor varies from 9 to 46 % of 6PFS (Table 10.2). It implies that metronomic treatment may serve as a useful platform for combination strategies in certain CNS tumors. Most newly developed antiangiogenic drugs remain in clinical trials, and these drugs can be combined with metronomic schedule of cytotoxic agents, when they eventually become available. These regimens may also present potential problems and challenges in terms of appropriate experimental study design and clinical testing. Nonetheless, future large phase III trials designed with the metronomic concept can clarify the benefit of this metronomic schedule.

Table 10.2 Studies reporting efficacy of metronomic chemotherapy in brain tumor

Studies	Year	Treatment modalities	*N*	Target	Efficacy (6PFS, OS)	Toxicities (grade 3 or 4)
Needle et al. [39]	1997	Etoposide 50 mg/m^2/day for 21 days every 28 days	28	Recurrent brain tumor (heterogeneous)	NA	Neutropenia (5), grade 4 thrombocytopenia (1)
Mulne et al. [40]	2000	MTX 7.5 mg/m^2 every 6 h for 8 doses weekly	78	Astrocytoma, malignant glioma, medulloblastoma, brainstem tumor, ependymoma, and miscellaneous	NA	Mucositis (24), leukopenia (28), neutropenia (25), thrombocytopenia (21), anemia (4), transamine elevation (7), neurologic (6)
Tuttenberg et al. [36]	2005	TMZ 10 mg/m^2 + rofecoxib 25 mg/d	13	Newly diagnosed GBM	PFS 8 months OS 16 months	Ischemic bowel perforation (1)
Brandes et al. [25]	2006	TMZ 75 mg/m^2/day for 21 days every 28 days	33	Recurrent GBM (chemonaive)	6PFS 30.3 % 1 year OS 38 %	Lymphopenia (8), neutropenia (4), thrombocytopenia (1), anemia (1), nausea (1), constipation (1)
Baruchel et al. [41]	2006	TMZ 50–75 mg/m^2/day	28	Recurrent pediatric brain tumors	6PFS 15 % OS 20.6 week	Lymphopenia and thrombocytopenia (5)
Kesari et al. [21]	2007	Etoposide (35 mg/m^2 daily for 21 days), alternating every 21 days with cyclophosphamide (2 mg/kg daily for 21 days) + with daily thalidomide and celecoxib	48	Recurrent GBM (28), recurrent grade III glioma (20)	GBM: 6PFS 9 %, OS 21 weeks Grade III glioma: 6PFS 26 %, OS 41.5 weeks	Colitis (2), constipation (5), leukopenia (13), neutropenia (10)
Clarke et al.[37]	2009	TMZ 50 mg/m^2/day	43	Newly diagnosed GBM	6PFS 46 % OS 15.1 months	Lymphopenia (61 %), leukopenia (14 %), elevation of aminotransferase (18 %)

(continued)

Table 10.2 (continued)

Studies	Year	Treatment modalities	*N*	Target	Efficacy (6PFS, OS)	Toxicities (grade 3 or 4)
Reardon et al. [22]	2009	Bevacizumab + metronomic etoposide	59	Recurrent GBM (27) grade III glioma (32)	6PFS 40.6/44.4 %	Neutropenia (24 %), thrombosis (12 %)
Perry et al. [26]	2010	TMZ 50 mg/m^2/day	116	Recurrent GBM (88), Recurrent grade III glioma (28)	6PFS 28.3 % 1-year OS 14.8–28.6 %	Lymphopenia (15.8 %), nausea/vomiting (8), fatigue (7), hyperglycemia (3)
Kong et al. [24]	2010	TMZ 50 mg/m^2/day	38	Recurrent GBM	6PFS 32.5 %, 6 months OS 56 %	Lymphopenia (3), neutropenia (1)
Sharp et al. [44]	2010	TMZ 85 mg/m^2/day with RT followed by TMZ 85 mg/m^2/day	15	Newly diagnosed brainstem glioma	6PFS 33 ± 12 % OS 9.8 months	Induction step: lymphopenia (13), leukopenia (1), neutropenia (1), thrombocytopenia (1)
Brada et al. [30]	2010	TMZ-21 (100 mg/m^2 for 21 days)	111	Recurrent GBM (83) Recurrent grade III glioma (28)	6PFS 36.2 % OS 6.6 months	Thrombocytopenia (11), neuropathy (15), leucopenia (9), neutropenia (7)
Stockhammer et al. [29]	2010	TMZ 20 mg/m^2 + celecoxib 200 mg	28	Recurrent GBM	6PFS 43 % 6 months OS 86 %	No grade 3/4 toxicity
Addeo et al. [45]	2012	TMZ 75 mg/m^2/day + WBRT + vinorelbine 70 mg/m^2	36	Brain metastasis from breast cancer	Median PFS 8 months	Neutropenia (1), toxicity (1), anemia (1), vomiting (1)

Peyrl et al. [42]	2012	Thalidomide, celecoxib, etoposide, cyclophosphamide	16	MB (7), PNET (2), ATRT (7)	NA	Neutropenia (14/16), lymphopenia (16/16), severe pneumonia (3/16), proteinuria (2/16) Peripheral neuropathy (7/16), hypothyroidism (6/16)
Robison et al. [43]	2013	21-day cycles of cyclophosphamide and etoposide, with thalidomide, celecoxib, and fenofibrate	69	High-grade glioma (21), ependymoma (19), low-grade glioma (12), medulloblastoma/ primitive neuroectodermal tumor (PNET, 8), miscellaneous	27-week PFS 25 % 27-week OS 60 %	Neutropenia (32 %), anemia (22 %), thrombocytopenia (5 %)
Omuro et al. [27]	2013	TMZ 50 mg/m^2/day	37	Recurrent GBM (37) grade III glioma (10)	6PFS 19 % OS 19 months	Lymphopenia (11), thrombocytopenia (1)
Gilbert et al. [38]	2013	TMZ 75–100 mg/m^2 for 21 days every 28 days	422	Newly diagnosed GBM	PFS 8.8 months OS 16.8 months	Leukopenia (36), neutropenia (36), lymphopenia (107), fatigue (33), nausea (8)

MB medulloblastoma, *PNET* primitive neuroectodermal tumor, *GBM* glioblastoma, *6PFS* 6-month progression-free survival, *OS* overall survival, *TMZ* temozolomide, *MTX* methotrexate, *WBRT* whole-brain radiotherapy

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Metronomic Chemotherapy in Pediatric Malignancies

11

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Abstract

Low-dose metronomic (LDM) chemotherapy, the frequent and continuous use of low doses of conventional chemotherapeutics, is an emerging alternative to conventional chemotherapy. Several pediatric preclinical solid tumor models are supporting the clinical development of this new therapeutic modality in pediatric cancer. Maintenance low dose chemotherapy has proven its benefits in increasing overall survival in several pediatric cancer. This chapter is reviewing the current knowledge of pediatric metronomic chemotherapy and potential for future development as cytotoxic agents or in combination with targeted therapy including its potential application in emerging countries.

11.1 Introduction

According to the WHO mortality report in 2008, cancer is the leading cause of disease-related death among children 5–14 years of age in high-income countries. Although communicable diseases remain the most common cause of death in low- and middle-income countries, because of high population density, 84 % of all children affected with cancer live in those countries [1, 2].

Conventional therapies have been effective in decreasing overall mortality rate from pediatric cancer; however, the prognosis remains poor for a subset of leukemias and lymphomas and metastatic solid tumors such as Ewing sarcoma, rhabdomyosarcoma, osteosarcoma, and neuroblastoma. Therefore, novel therapeutic approaches for these tumors should be explored, particularly in the setting of minimal residual disease that is associated with high risk of relapse and poor prognosis.

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11.2 Conventional Chemotherapy

Cytotoxic antiproliferative agents are the mainstay of conventional chemotherapy regimens. In conventional chemotherapy, maximum tolerated dose (MTD) of cytotoxic drugs is used to kill the tumor cells. This approach has improved the cure and survival rates for different types of pediatric cancer; however, short- and long-term adverse effects of high doses of cytotoxic agents are of considerable importance, especially in young survivors of pediatric cancer. Acute adverse effects of conventional chemotherapy are mainly due to nonspecific effect of the cytotoxic drugs on the proliferating cells. Seventy percent of pediatric cancer survivors experience long-term complications of conventional chemotherapy and almost 40 % of them suffer from life-threatening or debilitating complications [3]. Eighty-seven percent of childhood cancer survivors report multiple symptom classes that impair their health-related quality of life [4]. In addition, most solid tumors are composed of heterogeneous subpopulations of cells with different cell kinetics, metastatic characteristics, and angiogenic and invasive potential [5]. Therefore, their response to conventional chemotherapy varies widely. Despite the advances in conventional therapeutic approaches, the overall survival rate of some of the pediatric solid tumors such as high-risk neuroblastoma and metastatic sarcomas has not improved since a few decades ago.

11.3 Minimal Residual Disease

Minimal residual disease (MRD) is characterized by the presence of a small number of cells unaffected by therapy. MRD is an independent prognostic factor of poor response and a predictor of relapse in hematologic malignancies, neuroblastoma, and rhabdomyosarcoma [6–8]. Conventional chemotherapeutic approaches fail to inhibit a group of tumor cells that either escape therapy by hiding in sites characterized by poor oxygenation or drug penetration or develop resistance to chemotherapeutic drugs [9]. Therefore, relapsed tumors respond poorly to conventional chemotherapy.

It has been postulated that tumor-initiating cells (TICs), which are known to generate the bulk of the tumor through their self-renewal and extensive proliferation capacities might present as MRD [10–12]. TICs are resistant to chemotherapy and can survive as MRD in the primary location or metastatic organs [13, 14]. Although the concept of cancer stem cells was first explored in hematologic malignancies, further studies led to the identification of TICs in solid tumors. Hsu et al. recently reported a distinct subpopulation in neuroblastoma with stem cell-like phenotype and high tumorigenic potential [15].

11.4 Maintenance Therapy

Maintenance therapy has been used in pediatric malignancies for many years. The wide acceptance of the concept of maintenance therapy in pediatric malignancies is reflected in the standard protocols of ALL, where maintenance regimen is continued

for 2–3 years and consists of daily oral mercaptopurine, weekly methotrexate, vincristine, and corticosteroids [3]. Maintenance therapy with 13-cis-retinoic acid after myeloablative chemotherapy has improved the overall survival in neuroblastoma [16]. The goal of maintenance therapy is to prevent relapse by inhibiting the progression of MRD. Since maintenance therapy is administered in long term and usually in heavily pretreated patients, it should be minimally toxic. It is also crucial to avoid regimens, which have cross-resistance with previously administered drugs. The efficacy of maintenance therapy in improving survival rate was confirmed by a stage IV pediatric soft-tissue sarcoma trial comparing the oral maintenance regimen (consisting of trofosfamide + etoposide and trofosfamide + idarubicin) with high-dose therapy (thiotepa + cyclophosphamide and melphalan + etoposide). Patients who received maintenance therapy showed a survival rate of 57.8 % after 57.4 months versus 24.4 % in high-dose group [17].

11.5 Angiogenesis

Dr. Folkman first described the theory of tumor angiogenesis in 1971. He proposed that the size of the tumor is limited by its ability to develop new vasculature [18]. In addition to tumor growth, angiogenesis is required for metastatic spread and progression of tumor cells, as blood is the most common media to deliver tumor cells to other organs [19].

The mechanisms of tumor angiogenesis are varied. In sprouting angiogenesis, endothelial cells from preexisting blood vessels proliferate and migrate into tumor tissue to form the tumor vasculature. Intussusception refers to the process of the division of preexisting capillary plexus, without actual mitosis of endothelial cells [20]. In vasculogenesis endothelial progenitor cells are released from bone marrow and recruited by tumor tissue to form the new blood vessels [21]. Stromal Cell-Derived Factor-1 α and vascular endothelial growth factor (VEGF) are cytokines that facilitate the mobilization of bone marrow progenitors.

Tumor hypoxia simulates the angiogenic mechanisms. High turnover of tumor cells and abnormal architecture of tumor vasculature compromises oxygen and nutrient delivery to cells located far from the capillaries [22]. Under the hypoxic condition, stabilized HIF1-α forms a transcriptionally active complex (HIF1) with HIF1-β in the nucleus. HIF1 is a transcription factor for number of genes, involved in angiogenesis, cellular adaptation to hypoxia, and apoptosis [23]. VEGF is the most specific and critical angiogenic factor that is induced by HIF1-α.

Angiogenesis plays an important role in majority of pediatric cancers such as leukemia, CNS tumors, neuroblastoma, and pediatric sarcomas [24–28]. Inhibition of angiogenesis is therefore an effective maintenance therapy to control the growth of tumor or MRD.

Since the concept of tumor angiogenesis was suggested, several drugs with anti-angiogenic properties were studied such as endogenous antiangiogenic molecules (angiostatin, endostatin) and endothelial growth inhibitors (TNP-470, thalidomide), VEGF tyrosine kinase inhibitors and receptors, MMP inhibitors, and inhibitors of

vascular adhesion molecules [29]. Antiangiogenic agents are divided into two main categories based on their mechanism of action. Direct antiangiogenic drugs exert their effect directly on the endothelial cells, while indirect agents target growth factors or receptors involved in endothelial stimulation [30–35].

VEGF signaling pathway is the major pathway in tumor angiogenesis. Therefore, inhibition of VEGF pathway is the focus of most of the antiangiogenic strategies. Bevacizumab, a VEGF-neutralizing monoclonal antibody, was approved by FDA for colorectal cancer in 2004 [36]. Although it has not been yet approved for pediatric cancer, bevacizumab has been shown to delay tumor progression in an experimental metastatic neuroblastoma mice model [37]. Later, FDA also approved small-molecule receptor tyrosine kinase inhibitors (RTKIs) such as sunitinib, sorafenib, pazopanib, and axitinib, which inhibit VEGFR autophosphorylation [36]. In studies conducted by Pediatric Preclinical Testing Program (PPTP), pazopanib delayed the tumor growth and improved the survival in mice models of rhabdomyosarcoma and Ewing sarcoma [38]. Kumar et al. showed the efficacy of LDM topotecan + pazopanib in delaying tumor growth and enhancing the survival of neuroblastoma-bearing mice [39, 40].

11.6 Low-Dose Metronomic Chemotherapy

Low-dose metronomic (LDM) chemotherapy refers to the chronic administration of minimally toxic doses of cytotoxic agents without prolonged drug-free breaks. In 1991, Kerbel suggested that conventional chemotherapy drugs show antiangiogenic effects at low doses [41]. Klement et al. proved the efficacy of LDM vinblastine in neuroblastoma mouse model in 2000 [42]. Browder et al. could demonstrate the effectiveness of LDM cyclophosphamide in the xenograft models of breast cancer and Lewis lung carcinoma derived from cell lines, which were resistant to the same drugs [43]. Neoangiogenesis is a target for low-dose metronomic chemotherapy. Studies have shown that low doses of antiproliferative cytotoxic drugs inhibit tumor progression by antiangiogenic mechanism [42, 43].

LDM chemotherapy as a new therapeutic strategy has been explored in pediatric malignancy.

11.7 Preclinical Studies of LDM in Pediatric Tumor Models

Preclinical studies have provided valuable information about the efficacy and adverse effects of LDM chemotherapy in pediatric tumor models. In 2000, Klement et al. showed that in vitro low-dose vinblastine was more toxic to human umbilical vein endothelial cells (HUVEC) than to neuroblastoma cells and in vivo it caused a significant tumor growth delay and reduction in tumor perfusion [42]. In a study on Wilms tumor, topotecan reduced the size of the tumor at doses as low as 0.36 mg/kg with no observable adverse effects [44]. McCrudden et al. demonstrated growth inhibition and antiangiogenic effects of metronomic topotecan (0.36 mg/kg IV,

5 days/week for 6 weeks) in hepatoblastoma xenograft models [45]. The combination of conventional and metronomic scheduling of cytotoxic agents was shown to reduce tumor volume in osteosarcoma-bearing rat models [46]. Preclinical studies showed the effectiveness of extended low-dose topoisomerase I inhibitors against melphalan- and vincristine-resistant pediatric solid tumor xenografts [47]. Following the positive preclinical results, pharmacokinetically guided dosing schedule of topotecan was used in clinical studies involving pediatric solid tumors [48–50]. Later, Kumar et al. showed the superior efficacy of a combination of metronomic administration of topotecan and pazopanib over a single agent in either neuroblastoma, osteosarcoma, or rhabdomyosarcoma subcutaneous xenograft models. In comparison to single agents, the combination demonstrated enhanced antitumor activity and significantly increased the survival [39].

An international phase I clinical study of low-dose metronomic topotecan and pazopanib is about to start recruiting pediatric patients with recurrent or refractory solid tumors including CNS tumors (TOPAZ study).

Marimpietri et al. conducted in vitro and in vivo studies to investigate the antiangiogenic effects of low-dose vinblastine and rapamycin on neuroblastoma. They concluded that either agent alone could inhibit the growth of endothelial cells and the combination showed a significant synergistic effect [51]. Phase I clinical trial of the combination of weekly vinblastine and daily oral sirolimus (mammalian target of rapamycin inhibitor) for pediatric recurrent or refractory solid tumors showed the safety, clinical efficacy, and antiangiogenic properties of this combination [52]. This study along with other studies combining drugs to maximize the antiangiogenic effects signifies the importance of designing LDM regimens that could inhibit different mechanisms of angiogenesis.

11.8 Clinical Studies of LDM in Pediatric Malignancies

The number of clinical studies of LDM in pediatric malignancies is limited, but promising results have been achieved. Almost all pediatric studies have been conducted on metastatic or refractory tumors.

Some of the clinical trials have only used conventional cytotoxic agents with metronomic scheduling in pediatric malignancies. Fousseyni et al. showed the efficacy of a metronomic chemotherapeutic regimen consisting of vincristine, cyclophosphamide, and methotrexate in 12 children with refractory cancer (six cases of Wilms tumor, five cases of retinoblastoma, and one case of metastatic neuroblastoma). Disease stabilization was achieved in 7 patients (58 %) and 3 of them remained stable for at least 6 months posttreatment [53]. In another study on stage IV soft-tissue sarcoma, patients treated with low-dose metronomic cycles of trofosfamide, idarubicin, and etoposide showed better overall survival rate (0.52 + 0.14) compared to patients receiving high-dose chemotherapy (0.27 + 0.13) [17]. A phase II trial with metronomic thalidomide-carboplatin-vincristine-fluvastatin in pediatric brainstem tumors showed significant reduction in tumor volume after treatment. Partial response was observed in 7 out of 9 patients [54].

Table 11.1 Recent clinical trials of LDM chemotherapy in pediatric malignancies

Cytotoxic drug	Disease
Trofosfamide/idarubicin/etoposide	Embryonal rhabdomyosarcoma (higher event-free survival and lower relapse when high-dose chemotherapy was followed by maintenance regimen) [59]
Vincristine/cyclophosphamide/methotrexate	Pediatric refractory cancers (well tolerated and associated with disease stabilization) [53]
Topotecan (0.8 mg/m^2/day) for 21 days, repeated every 28 days	Recurrent pediatric brain tumor (ependymoma, high- grade glioma, brainstem glioma, and primitive neuroectodermal tumor). Regimen was safe in all patients. Oral topotecan achieved remission in 2 out of 25 patients who are alive 7 and 9.5 years after therapy. Both patients had disseminated medulloblastoma at study entry [55]
Temozolomide	Pediatric brainstem glioma (median duration was three cycles of 6 weeks' therapy (85 mg/m^2 daily); the first cycle was given with induction radiotherapy); median overall survival, 9.8 months; prolonged hematologic toxicity was observed [60]
Temozolomide	Recurrent pediatric brain tumors (of 28 patients, 2 complete response and 2 partial response), metronomic scheduling was associated with higher cumulative drug exposure and lower grade 3/4 toxicity compared with conventional schedule [61]

Minturn et al. demonstrated the efficacy of oral metronomic topotecan in recurrent childhood brain tumors. Disease stabilization and partial response were observed in 5 (20 %) and 2 (8 %) out of 25 patients, respectively [55].

Metronomic temozolomide has been used in combination with radiotherapy in children ($n=2$) and adults ($n=3$) with recurrent medulloblastoma. Local control was achieved in one of two pediatric patients who later developed relapse in another location under treatment with Choi protocol. Local relapse occurred in the other pediatric patient 10 months after reirradiation. The patient was reported to have stopped metronomic temozolomide earlier than planned. None of the patients showed neurological toxicity [56]. Sondhi et al. reported a case of complete remission of relapsed medulloblastoma with extensive osteosclerotic bony metastasis in a 14-year-old boy with LDM chemotherapy consisting of etoposide, cyclophosphamide, and zoledronic acid administered for 18 months. Complete response was maintained for >24 months (by the time the paper was written) with good quality of life [57].

In addition to more common cancers, metronomic chemotherapy has been associated with good results in less common pediatric malignancies. Chaudhary et al. reported the complete remission of a malignant peripheral nerve sheath tumor (MPNST) with metronomic chemotherapy. A combination of metronomic oral etoposide, cyclophosphamide, and prednisolone was administered successfully to a 10-year-old male with recurrent MPNST. Complete remission was sustained 20 months after the sessions of metronomic therapy [58]. Table 11.1 presents a summary of some of the recent LDM chemotherapy trials.

11.9 Combining Cytotoxic and Antiangiogenic Agents in LDM Chemotherapy

Tumor endothelial cells are susceptible to metronomic scheduling of conventional cytotoxic agents; however, upregulation of VEGF by endothelial cells can negate the antiangiogenic effects of LDM chemotherapy [62]. In addition to its growth factor effect, VEGF acts as a survival/antiapoptotic agent for endothelial cells through different mechanisms such as upregulation of antiapoptotic protein survivin, Bcl-2, and A1 in endothelial cells [63–65].

The combination of low-dose cytotoxic drugs with antiangiogenic agents has been studied in a few clinical trials in pediatric population with promising results.

A combination of temozolomide with celecoxib, 13-cis-retinoic acid, and etoposide in COMBAT (combined oral maintenance biodifferentiating and antiangiogenic therapy) protocol has been studied in 22 heavily pretreated children with relapsed solid tumors. Clinical response was observed in 9 of 14 children (64 %) with progressive disease. Patients showed good tolerance and compliance for oral medications. Side effects were minimal and responded well to dose modification or local therapy [66]. COMBAT regimen (low-dose daily temozolomide, etoposide, celecoxib, vitamin D, fenofibrate, and retinoic acid) was later used in another study achieving a 2-year overall survival in 43.1 % of patients with advanced pediatric malignancies [67].

Andrè et al. conducted a pilot study to evaluate the feasibility of a metronomic 4-drug regimen in pediatric patients with refractory or relapsing tumors. The combination consisted of vinblastine, cyclophosphamide, methotrexate, and daily celecoxib in cycles of 56 days. One objective response and 4 (25 %) disease stabilization were observed among 16 patients. Tolerability was acceptable. Interestingly, they reported reduced pain in 11 patients shortly after initiation of LDM chemotherapy [68].

Stempak et al. studied the combination of celecoxib and LDM vinblastine or cyclophosphamide in refractory pediatric solid tumors. The combination was well tolerated and 4 of 33 patients (13 %) experienced durable stable disease (28–78 weeks) [69].

In a recent phase II trial, Robison et al. evaluated the efficacy of a multi-agent metronomic therapeutic regimen consisting of celecoxib, thalidomide, and fenofibrate, with alternating 21-day cycles of low-dose cyclophosphamide and etoposide in children with recurrent or progressive disease. Favorable outcome including partial response and stable disease was reported for ependymoma and low-grade glioma. High-grade glioma and bone tumors responded poorly to treatment. The 27-week overall survival rate was 60 %. Grade 4 neutropenia (32 %) was the most common toxicity [70].

Peyrl et al. reported the therapeutic results of an antiangiogenic multidrug combination regimen consisting of bevacizumab, thalidomide, celecoxib, fenofibrate, etoposide, and cyclophosphamide and additional intraventricular therapy (etoposide and liposomal cytarabine) in children with recurrent embryonal brain tumors. Three complete and two partial responses were observed in five evaluable patients with medulloblastoma. Disease progression was seen in all patients with CNS primitive

Table 11.2 Recent clinical trials involving combination of LDM chemotherapy with antiangiogenic drugs in pediatric malignancies

Drug	Combination/disease	Major observation
Cyclophosphamide	Celecoxib (non-Hodgkin's lymphoma) [74]	37 % response and 22 % SD
Vinblastine or cyclophosphamide	Celecoxib (pediatric recurrent solid tumors) [69]	13 of 33 patients had stable disease
Vinblastine	Sirolimus (pediatric recurrent or refractory solid tumors) [52]	Of 11 patients, 1 had partial response and 3 stable disease
Cyclophosphamide	Zoledronic acid (recurrent/refractory neuroblastoma) [73]	Of 21 patients, 1 had partial response and 9 stable disease
Etoposide and cyclophosphamide	Celecoxib (pediatric and adolescent refractory cancer) [75]	7 (41 %) of 17 patients had stable disease Therapy reduced antalgic needs in 4 (24 %) patients
4-drug regimen	Weekly vinblastine, daily cyclophosphamide, twice-weekly methotrexate, and daily celecoxib In refractory and relapsed pediatric tumors [68]	56-day (8-week) treatment was well tolerated and achieved disease stabilization
5-drug regimen	Daily oral thalidomide and fenofibrate, twice-daily oral celecoxib, and alternating 21-day cycles of low-dose oral etoposide and cyclophosphamide in recurrent or progressive disease [70]	27-week treatment duration treatment was well tolerated Clinical benefit was seen in ependymoma and low-grade glioma

neuroectodermal tumors (CNS PNET, $n=4$) and one out of seven patients with medulloblastoma. Six-month overall survival was 100 % and 75.0 ± 22 % for medulloblastoma and CNS PNET, respectively [71].

A Children's Oncology Group (COG) phase II study (NCT00061893) demonstrated the feasibility of combination of standard multi-agent chemotherapy with low-dose vinblastine and celecoxib in 35 patients with metastatic Ewing sarcoma. Patients did not show excessive neurologic complications, infections, mucositis, and GI bleeding; however, the frequency and severity of pulmonary toxicity and hemorrhagic cystitis in patients who received radiation were unexpectedly high. The 24-month event-free survival of 71 % for patients with isolated pulmonary metastasis was higher than historical controls [72].

The combination of metronomic chemotherapy with non-antiangiogenic agents has been also studied. Russell et al. studied the efficacy of zoledronic acid with metronomic cyclophosphamide in 20 patients with recurrent/refractory neuroblastoma. One partial response and 9 stable disease responses (maintained for 2–12 months) were observed. The combination was well tolerated [73].

Table 11.2 summarizes some of the recent clinical trials of LDM chemotherapy with the combination of cytotoxic and antiangiogenic drugs.

11.10 Advantages of LDM

Cytotoxic drugs in high doses affect both tumor cells and normal proliferating cells. Therefore, most of the acute side effects of conventional chemotherapy are related to its cytotoxic properties. Lower doses of cytotoxic drugs in LDM result in less acute toxicity effects [76, 77]. Furthermore, the main target of LDM is endothelial cells in contrast to conventional chemotherapy where killing tumor cells is the ultimate goal. In a pioneering preclinical study conducted by Klement et al., it was observed that the sensitivity of human umbilical vein endothelial cells (HUVEC) to low-dose vinblastine was significantly higher than neuroblastoma cell lines [42].

Importantly, tumors resistant to high doses of a cytotoxic agent in conventional chemotherapy might be still sensitive to LDM scheduling of the same drug [43]. Furthermore, LDM can enhance the chemosensitivity of endothelial cells contrary to MTD (where cross-resistance between paclitaxel and vinblastine has been demonstrated) [78].

Few studies focused on the feasibility of metronomic chemotherapy in low-income countries. Inexpensive anticancer drugs can be used to design metronomic chemotherapy cycles. Furthermore, less acute toxicities associated with lower doses of cytotoxic agents in LDM chemotherapy are advantageous in areas with limited medical resources. Disease stabilization achieved by a metronomic regimen consisting of vincristine, cyclophosphamide, and methotrexate in children with different types of refractory tumors in Mali confirmed the feasibility and cost-effectiveness of this approach in low-income countries [53]. In "Metro-Mali-02" study, the same combination plus valproic acid resulted in long-lasting partial response (2 years) in two out of seven children, one with metastatic neuroblastoma and the other with retinoblastoma [79].

In low-income countries where curative or novel experimental treatments are not accessible for children with progressive cancer, low-cost maintenance therapy with inexpensive cytotoxic drugs is a viable option.

11.11 Limitations of LDM

11.11.1 Biomarkers

Preclinical studies and clinical trials have increased our knowledge of LDM chemotherapy and its clinical applications. Contrary to conventional chemotherapy, LDM affects tumor indirectly through antiangiogenesis. Therefore, biomarkers required for monitoring the efficacy and progress of treatment with LDM is different from conventional chemotherapy. Response Evaluation Criteria In Solid Tumors (RECIST), which evaluates the efficacy of therapy by tumor burden, does not reflect the response of tumors to antiangiogenic effects of LDM chemotherapy accurately [80]. Hence, surrogate markers should be explored to monitor tumor's response to

cytostatic effects of LDM. Proangiogenic and antiangiogenic growth factors and cytokines such as VEGF, basic fibroblast growth factor (bFGF), soluble vascular cell adhesion protein 1 (sVCAM-1), endostatin, and thrombospondin-1 (TSP-1), which were initially thought to correlate with the clinical benefits of LDM chemotherapy, failed to show consistent results in further studies [81]. In a pilot pharmacokinetic study of celecoxib and low-dose metronomic vinblastine or cyclophosphamide for pediatric patients with recurrent solid tumors, Stempak et al. showed that VEGF, bFGF, sVCAM-1, endostatin, and TSP-1 did not correlate with disease progression or maintenance of stable disease [69]. In a phase I trial of bevacizumab in refractory pediatric solid tumors, it was shown that baseline VEGF, TSP-1, bFGF, CEC, and CEP were not correlated with clinical benefit. However, researchers observed increased levels of mature CECs with treatment [82]. In a multicenter study of metronomic temozolomide combined with radiotherapy in pediatric patients with brainstem glioma, the decreasing trend of VEGF and endoglin was observed during the first two cycles of therapy. The decreasing trend of VEGF was associated with longer event-free survival [60]. In a phase I pharmacokinetic and pharmacodynamic study of pazopanib, it was shown that therapy significantly reduces plasma soluble VEGFR-2 and endoglin. A lower baseline plasma level of VEGF and placental growth factor was associated with clinical benefit [83].

Clinical trials in adult malignancies suggested a correlation between increasing levels of circulating endothelial cells (CECs) and disease progression [84]. The utility of CEC as a biomarker to monitor the antiangiogenic effects of LDM regimens was further explored in breast cancer, lymphoma, and GI stromal tumor with promising results [74, 85–87].

Higher levels of CEP have been detected in patients with pediatric malignancies compared to healthy controls. Children with metastatic disease show higher levels of CEP in comparison to localized disease [88].

In a preclinical study of aggressive pediatric solid tumors, it was demonstrated that the combination of metronomic oral topotecan and pazopanib significantly decreased viable CEC, viable CEP, and microvessel density [39]. In a clinical study with metronomic cyclophosphamide and celecoxib in non-Hodgkin's lymphoma pediatric patients, CECs, CEPs, and VEGF remained low in responders after 8.4 months of follow-up [74]. Although CEC and CEP have shown promising results in adult malignancies, future preclinical and clinical studies in pediatric malignancies are required to address the utility of these cellular markers for monitoring tumor response to LDM therapy. Imaging is an alternative option to evaluate the response of tumor to antiangiogenesis. It has been demonstrated that changes in blood volume, blood flow, and vascular permeability correlate with the efficacy of antiangiogenic treatment [89].

11.11.2 LDM Cycles: Rationale and Design

Different drugs and drug combinations have been used in the preclinical studies and clinical trials of LDM in pediatric malignancies. The following criteria are used to choose a drug as a potential candidate for LDM chemotherapy: (a) it should be

nonoverlapping and minimally toxic, (b) it could be administered orally, (c) it should have established antiangiogenic and/or immunostimulant effects, and (d) it should have low probability of developing drug resistance [90].

Pediatric patients with high-grade or refractory solid tumors are considered to benefit from LDM therapy. The type of tumor, its biological properties, and its clinical setting should be carefully considered before choosing a drug or drug combination for clinical trials of LDM chemotherapy in pediatric population. The optimal dose for the best therapeutic response is another challenge in developing LDM regimens for clinical trials. Contrary to conventional chemotherapy where maximally tolerated doses of drugs are chosen for the best clinical outcome, LDM therapy relies on the cytostatic effects of low doses of chemotherapeutic or antiangiogenic agents. Pharmacodynamic biomarkers are required to establish the optimal dose of antiangiogenic agents for LDM regimens. It has been speculated that LDM chemotherapy hinders mobilization of CEP from the bone marrow. Hence, CEP has been successfully used as a pharmacodynamic biomarker to provide information about optimum biological dose of metronomic cyclophosphamide, vinblastine, vinorelbine, and cisplatin in mice models of breast cancer, melanoma, and erythroleukemia [91].

In addition to drug and dose selection, defining clinical end points is an important step in designing and monitoring LDM therapy. Event-free survival and response rate that are commonly used in conventional chemotherapy have been employed in LDM clinical trials. Disease stabilization and good quality of life are other clinical benefits of LDM chemotherapy that could be incorporated into trials as clinical end points.

The duration of LDM chemotherapy in pediatric patients is another important question that should be answered. Sudden discontinuation versus gradual tapering of LDM chemotherapy should be compared in well-designed and closely monitored clinical trials.

Acute toxicity of LDM chemotherapy is lower than conventional chemotherapeutic approaches [76, 77, 92]. However, chronic administration of cytotoxic agents and subsequent accumulative doses might result in adverse events. Long-term side effects of LDM chemotherapy is therefore a concern that should be addressed in future studies.

Resistance to LDM chemotherapy emerged despite the initial assumptions about the genetic stability of tumor endothelial cells [62]. Resistance mechanisms are evoked in response to tumor microenvironment changes caused by antiangiogenic treatment. Upregulation of angiogenic factors [93, 94], involvement of bone marrow-derived cells [95, 96], and pericyte coverage [97] are some of the known mechanisms of resistance to LDM chemotherapy.

11.12 Future Direction

LDM chemotherapy is an alternative to conventional chemotherapy, which has shown promising results in preclinical and clinical studies of pediatric malignancies. However, more clinical trials are required to assess the efficacy and safety of LDM in pediatric population. Appropriate clinical end points should be defined with respect to antiangiogenic effects of LDM therapy. Studies should be conducted

to validate biomarkers for evaluating the activity of treatment. Long-term side effects of LDM are of great importance and should be addressed in future studies. It should be emphasized that the goal of LDM is to be integrated into cancer maintenance therapy to control MRD and provide a good quality of life for patients living with cancer.

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Metronomic Chemotherapy in Hematological Malignancies

12

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Abstract

Targeting tumor microenvironment and angiogenesis is a novel therapeutic strategy in hematological malignancies. The antiangiogenic effects of chemotherapeutic agents can be optimized when administered metronomically, by providing low doses of chemotherapeutic drugs on a continuous schedule without extended drug-free intervals. Metronomic chemotherapy preferentially targets endothelial cells of the growing tumor neovasculature instead of tumor cells themselves and therefore can be particularly effective against multidrug-resistant tumors. Metronomic therapy may further enhance immune response by modulating antitumor NK/T-cell functions. The past decade saw an increasing appreciation of the pathogenic roles that tumor angiogenesis plays in hematological malignancies including leukemias, lymphomas, and multiple myeloma. Experimentation with a variety of antiangiogenesis modalities has shown encouraging efficacy with metronomic chemotherapy in these disease categories, with generally low toxicity and cost. With the growing availability of the target-specific biological agents, some of which are specific for antiangiogenesis, it is conceivable that metronomic chemotherapy, either alone or in combination with biologics, has therapeutic potential in frontline and maintenance setting, in addition to its traditional role of salvage option for relapsed diseases in hematological malignancies.

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12.1 Metronomic Chemotherapy

12.1.1 Definition

In contrast to the conventional "maximum tolerated dose" (MTD) chemotherapy, which provides bolus infusions of substantially dosed chemotherapeutic agents separated by 3–4-week-long breaks in between, metronomic chemotherapy refers to the administration of low doses of medications on a frequent or continuous schedule without extended drug-free breaks [1, 2]. The main targets of metronomic therapy are the endothelial cells and stromal cells of the growing tumor [3, 4]. Consequently, in addition to its reduced acute toxicity, metronomic therapy can be particularly efficacious against multidrug-resistant tumors. Another unique advantage of metronomic chemotherapy is the clinical feasibility of combining low-dose chemotherapy with many of the new targeted and biological compounds to augment antitumor effects, thus improving clinical response without added toxicity.

12.1.2 Therapeutic Mechanisms

12.1.2.1 Antiangiogenesis

The classic study by Browder and colleagues, which showed that chronic administration of low-dose cyclophosphamide induced apoptosis of tumor-associated endothelial cells, leading to tumor cell apoptosis and subsequent tumor growth inhibition and eradication, established the endothelial cells of the growing tumor microvasculature as the main targets of metronomic chemotherapy [5]. Subsequent analysis indicated that low-dose metronomic therapy with various chemotherapy compounds had direct and selective cytotoxicity against the cycling vascular endothelial cells compared to the tumor cells and stromal fibroblasts [6]. One of the antiangiogenesis mechanisms may involve the induction of thrombospondin-1 (TSP-1), a potent endothelial-specific inhibitor of angiogenesis. TSP-1 level was shown increased in tumor models with metronomic treatment, while the antiangiogenic and antitumor effects of low-dose continuous cyclophosphamide were abolished in TSP-1 null C57BL/6 mice [7]. In addition to targeting local endothelium preferentially, metronomic therapy also effectively suppressed the surge of bone marrow-derived endothelial cell mobilization, which typically followed conventional MTD therapy, thereby reducing rebound tumor angiogenesis [8]. Maximal suppression of circulating endothelial progenitors (CEP) was correlated to maximal antiangiogenesis and antitumor activity [9].

12.1.2.2 Immune Activation

It has been increasingly appreciated that metronomic chemotherapy plays an important role in inducing antitumor immune response [10]. In both preclinical and clinical studies, low-dose cyclophosphamide administration was associated with decreases in the number and functionality of the $CD4^+CD25^+$ regulatory T cells (Treg), which mediate tumor-induced immune tolerance [11]. Mechanistically, Treg

inhibition restored NK/T-cell functions and facilitated the recruitment and selection of latent higher-avidity effector T cells, allowing sustained antitumor immune responses [12, 13]. Oral topotecan has been shown to stimulate the expression of major histocompatibility complex (MHC) class I in tumor cells, thereby augmenting antitumor T-cell cytotoxicity [14]. Additional immune-enhancing activities associated with metronomic therapy include promoting the maturation of dendritic cells, maintaining the survival of memory T cells, and reducing immunosuppressive cytokines [15, 16].

It thus appears that metronomic chemotherapy may act through various mechanisms and on various components of the tumor microenvironment, including mediating direct endothelial apoptosis, modulating angiogenic balance with upregulation of antiangiogenic factors, suppressing mobilization of CEPs to block neoangiogenesis, and depleting T-regulatory cells to facilitate the restoration of antitumor immunity.

12.2 Pathological Angiogenesis in Hematological Malignancies

Angiogenesis, the formation of new blood vessels, has been shown to be critically important for the pathogenesis of hematological malignancies, including acute and chronic leukemia, lymphoma, multiple myeloma (MM), myelodysplastic syndrome (MDS), and myeloproliferative neoplasm (MPN). Targeting tumor microenvironment and angiogenesis is a novel therapeutic strategy in hematological malignancies. The antiangiogenic effects of chemotherapeutic agents can be optimized when administered metronomically [1].

12.2.1 Leukemia

Acute leukemias are clonal disorders of hematopoietic stem cells (HSC) with high proliferative potential. The survival and growth of the leukemic cells are dependent on the pro-angiogenic interplay between the vascular niche within the bone marrow (BM) microenvironment and the neoplastic clones. Specifically, blood vessel network within the bone marrow provides protective sanctuary for the leukemic cells [17], while leukemic cells stimulate angiogenesis by expression of vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) to enhance endothelial cell proliferation [18–22].

In vitro study demonstrated that AML blasts can enhance microvascular endothelial cell (MVCE) proliferation in transwell coculture, and the survival of these leukemia cells was further enhanced when cocultured with either endothelial or marrow stromal cells [18, 23]. In vivo study with human leukemia cells implanted in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice showed increasing bone marrow neovascularization [24]. In a cranial window implantation model in SCID mice, AML cells induced tortuous and

hyperpermeable neovascularization, bearing the hallmarks of tumor angiogenesis [25]. Therapeutic intervention in experimental leukemia models with anti-VEGF bevacizumab [26], anti-VEGFR2 antibody [27–29], or vascular disrupting agents [30], led to leukemia cell apoptosis, validating angiogenesis as therapeutic target. In human patients, BM microvessel density (MVD) was significantly increased in patients with ALL and AML compared to normal control [31]. Remission following therapy was associated with decreased MVD, while relapse was associated with rebound MVD [32].

12.2.2 Lymphoma

Non-Hodgkin lymphoma consists of a collection of complex disease subtypes driven by monoclonal proliferation of malignant B or T cells, which display a spectrum of clinical behaviors, ranging from indolent subtypes with growth latency measured in years to aggressive subtypes with progression measured in weeks to months [33]. Emerging evidence suggests divergent angiogenic patterns in disparate lymphoma subtypes. To date, several lines of evidence have pointed to the relevance of angiogenesis to lymphoma progression and clinical outcome. Firstly, increased production of pro-angiogenic growth factors by lymphoma tumor cells as well as infiltrating stromal cells and increased angiogenesis activity (by vessel counts) have been observed with histological progression of the lymphomas [34–36]. Secondly, expression of both VEGF and VEGF receptors by the tumor cells in a number of lymphoma subtypes suggests autocrine and paracrine pro-angiogenic survival mechanisms. Thirdly, large-scale gene expression studies in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) have demonstrated that genetic signatures expressed by stromal and infiltrating immune cells define distinct prognostic groups, with increased angiogenic activity associated with inferior outcome in DLBCL [37, 38].

Lymphoma microenvironment has been increasingly recognized to influence angiogenesis and neoplastic progression. In human subjects, lymphoma-associated macrophages (LAM) have been implicated in aggressive disease and inferior outcome in follicular lymphoma [39]. Human non-Hodgkin lymphomas have been shown to have distinct perivascular patterns in disparate subtypes [40]. In aggressive subtypes of Burkitt's lymphoma and DLBCL, VEGF-producing $CD68^{+}VEGFR1^{+}$ myelomonocytic hematopoietic cells were closely associated with neovessels, lending structural and paracrine support to nascent vasculature, which was largely devoid of α-SMA^{+} pericyte coverage in response to rapid neoplastic growth. In contrast, the perivascular compartment in indolent CLL/SLL was marked by diffuse α-SMA^{+} pericytic coverage, leading to a more mature vascular composition. Our group recently showed that treatment with imatinib, a tyrosine kinase inhibitor that targets platelet-derived growth factor receptor β (PDGFRβ), depleted PDGFRβ + pericytes and disrupted microvascular angiogenesis in mouse models of human diffuse large B-cell lymphoma [41]. This ultimately translated into impaired lymphoma growth. This study provided proof of principal that targeting non-tumor

vascular cells within the lymphoma microenvironment can result in significant inhibition of lymphoma growth, providing the basis for more refined consideration of antiangiogenesis as a treatment strategy for lymphoma patients.

12.2.3 Multiple Myeloma

Multiple myeloma (MM) is a clonal disorder of proliferation of malignant plasma cells in the bone marrow, characterized by rising serum or urine monoclonal protein, and at least one of the symptoms of end-organ damage including osteolytic bone lesion, renal disease, anemia, and hypercalcemia. Bone marrow angiogenesis is a hallmark of disease progression and adverse outcome [42, 43]. Tumor-associated blood vessels within the bone marrow vascular niche have been shown to consist of loosely attached monolayer endothelial cells (ECs), often discontinuous with rare pericytes. The chaotic and variable blood flow leads to local hypoxia, stimulating further angiogenesis [44].

The degrees of MVD, pro-angiogenic cytokines (VEGF and VEGFR), and fibrosis in BM microenvironment of MM patients were significantly higher than those in monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM, two indolent forms of plasma cell dyscrasia [45]. The change from avascular phase to angiogenic phenotype was called "angiogenic switch" [46–49], which was supported in part by the pro-angiogenic interaction between MM-specific ECs (MMECs) and plasma cells (PCs). MMECs secreted CXC chemokines including IL-8, SDF1- α, and PC-growth factors including VEGF, FGF-1, MMP-9, whereas MM and stromal cells produced EC-survival factor such as VEGF [17, 44, 50, 51]. Biomarker studies have demonstrated that levels of circulating endothelial progenitor cells (EPCs) and circulating endothelial cells (CEC) correlated with MM progression and response to therapy with immunomodulatory compounds [52].

12.3 Application of Metronomic Chemotherapy in Hematological Malignancies

The principle of metronomic therapy has been practiced in oncology settings with variations on dosing schedule and drug sequence (Table 12.1).

12.3.1 Leukemia

12.3.1.1 Continuation Treatment in ALL

Remission maintenance therapy in acute lymphoblastic leukemia (ALL) is an example of metronomic treatment, which consists of weekly administration of methotrexate and daily administration of mercaptopurine [53]. The starting doses are 20–40 mg/m^2 for methotrexate and 50–75 mg/m^2 for mercaptopurine [54]. Comparing with different doses and schedules of maintenance treatment, continuous

Table 12.1 Metronomic regimens in hematological malignancies

Malignancy categories	Regimen	Disease	Phase and study	Sample size	Treatment efficacy				Reference
					ORR	CR	PR	Clinical outcome	
Leukemia	PET + ATRA	APL	Phase II	23	N/A	91.3 %	N/A	24-month EFS: 73 % 24-month OS: 84 %	[55]
	AraC + ATO	AML	Phase I/II	64	N/A	34 %	N/A	Mortality rate: 8 % within the first 4 weeks	[56]
	AraC + ATO	MDS	Phase I/II	49	17 %	17 %	0 %	Median DOR: 7.5 months Median OS: 8.4 months	[57]
Lymphoma	COPBLAM III	DLBCL	Phase II	51	96 %	84 %	12 %	Median F/U: 44 months 2-year PFS: 65 %	[58]
	Etoposide	Lymphoma (R/R)	Phase II	25	60 %	60 %	0 %	Median TTP: 5 months	[59]
	Vinblastine	ALCL (R/R)	Retro	30	83 %	83 %	N/A	36 % remained in CR at median F/U 7 years	[60]
	COX-2i and CTX	Aggressive NHL (R/R)	Phase II	35	37 %	6 %	31 %	Median F/U: 8.4 months Median PFS: 4.7 months Median OS: 14.4 months Median DOR: 8.2 months	[61]
	PEPC	MCL (R/R)	Retro	22	82 %	46 %	36 %	Median time on therapy: 17 months	[62]
	PEPC	Lymphoma (R/R)	Retro	75	69 %	36 %	33 %	Median time on therapy: 10 months	[63]
	RT-PEPC	MCL (R/R)	Phase II	22	73 %	32 %	41 %	Median F/U: 38 months Median PFS: 10 months	[64]
	Lomustine 50 mg/m^2 D1 VP16 100 mg/m^2 D1-3 CTX 50 mg/m^2 D22-26 Procarbazine 50 mg/m^2 D22–26	AIDS-NHL	Phase II	49	78 %	58 %	20 %	Median F/U: 8.2 months Median EFS: 7.9 months Median OS: 12.3 months	[65]

Multiple myeloma	CP CTX = 50 mg/day Pred = 15 mg/day	MM (R/R)	Retro	27	67 %	N/A	N/A	TTR: 2 months PFS: NR in responders, 4 months in nonresponders	[66]
	CTP CTX = 50 mg/day Tal = 200 mg/day Pred = 50 mg pod	MM (R/R)	Phase II	37	63 %	20 %	37 %	TTR: 3.6 months TTP: 13.2 months	[67]
	CBD CTX = 50 mg/day Bor = 1.3 mg/m^2[a] DEX = 20 mg/day[b]	MM (R/R)	Phase II	50	90 %	16 %	66 % (PR) 8 % (MR)	Median EFS: 12 months Median OS: 22 months	[68]
	CDT CTX = 300 mg/m^2/week Tal up to 300 mg/day DEX pulse	MM (R/R)	Phase II	52	90 %	17 %	62 % (PR) 11 % (MR)	Median F/U: 18 months 2-year OS: 73 % 2-year EFS: 34 % Median PFS: NR	[69]
	CyBorP CTX = 300 mg/m^2/week Bor = 1.3 or 1.5 mg/m^2 Pred = 100 mg pod	MM (R/R)	Phase I-II	37	92 %	54 %	31 % (PR) 7 % (MR)	1-year PFS: 83 % 1-year OS: 100 %	[70]
	CyBorD CTX = 300 mg/m^2/week Bor = 1.3 mg/m^2 DEX pulse	MM (New)	Phase II	33	88 %	39 %	61 % (VGPR)	23 underwent stem cell transplant Survival data N/A	[71]

AML acute myeloid leukemia, *MDS* myelodysplastic syndrome, *DLBCL* diffuse large B-cell lymphoma, *NHL* non-Hodgkin lymphoma, *MM* multiple myeloma, *Retro* retrospective, *R/R* relapsed/refractory, *TTP* time to progression, *TTR* time to response, *N/A* not available, *NR* not reached, *PET* prednisolone, etoposide, and 6-thioguanine (TG), *AraC* cytarabine, *ATO* arsenic trioxide, *CTX* cyclophosphamide, *TAL* thalidomide, *Pred* prednisone, *Bor* bortezomib, *COX-2i* COX-2 inhibitor, *PEPC* prednisone, etoposide, procarbazine, and etoposide

[a]Bortezomib was given at 1.3 mg/m^2 on days 1, 4, 8, and 11 for cycles 1–8 and then 1.3 mg/m^2 on days 1, 8, 15, and 22 for cycles 9–11

[b]Dexamethasone was given 20 mg/day on the day of bortezomib and the day thereafter

administration showed fewer relapse rates than intermittent pattern. The relapse rate increased if the maintenance treatment lasted less than 2 years.

12.3.1.2 Low-Dose Cytarabine

The combination of low-dose cytarabine up to 10 mg/m^2 given subcutaneously twice daily from days 1–14, together with arsenic trioxide (ATO) intravenously at 0.25 mg/kg on days 1–5 and 8–12, was studied in 64 elderly patients with AML [56]. The study subjects had high-risk features including 16 % secondary AML, 55 % unfavorable cytogenetics, 53 % poor performance status, and a median age of 71 years. Complete remission (CR) was achieved in 34 % patients. Among the CR patients, 30 % had secondary AML, 30 % had unfavorable cytogenetics, and 18 % had poor baseline performance status. The mortality rate in the first 4 weeks was 8 %, and hematological toxicities included neutropenic fever in 80 % of the patients.

A similar schedule of low-dose cytarabine and ATO was investigated in 49 patients with intermediate 2 to high-risk myelodysplastic syndrome (MDS) [57]. Eight patients (17 %) achieved CR. Median CR duration was 7.5 months. Median OS was 8.4 months, including 7.1 months in nonresponders, and 17.8 months in responders. Induction mortality was similar to the AML study [56], and other side effects were acceptable.

12.3.1.3 PET Oral Chemotherapy

Oral chemotherapy with prednisolone 40 mg/m^2/day, etoposide 50 mg/m^2/day, and 6-thioguanine (TG) 40 mg/m^2/day (PET) was given for 21 days as induction therapy with all-trans-retinoic acid (ATRA) to 23 patients with acute promyelocytic leukemia (APL) in India who did not have access to standard induction chemotherapy [55]. ATRA was given at 45 mg/m^2/day for 90 days. Responding patients were subsequently treated with three cycles of single-agent daunorubicin at 45 mg/m^2/day daily on days 1–3 every 21 days for consolidation, followed by six cycles of oral PET. ATRA was continued every 3 months for 15 days for a total of 18 months. Complete molecular remission was achieved in 91.3 % of the patients at a median of 40 days. EFS and OS at 24 months were 73 and 84 %, respectively. Treatment was outpatient based and well tolerated.

12.3.2 Lymphoma

12.3.2.1 Infusional COPBLAM III

Clinical precedents of metronomic therapy in lymphoma can be found in classical regimens such as COPBLAM III, which was first introduced in the 1980s. COPBLAM III (cyclophosphamide, infusional vincristine and bleomycin, prednisolone, doxorubicin, and procarbazine) incorporates nonmyelosuppressive continuous infusional vincristine and bleomycin designed to overcome drug resistance. In the initial study of 51 patients with large diffuse cell lymphoma, 84 % of patients achieved CR, of which 92 % were sustained at a median follow-up of 40 months. Pulmonary toxicity occurred in 39 % of patients [58, 72].

12.3.2.2 Etoposide

Single-agent etoposide was evaluated in a phase II study in 25 refractory lymphoma patients using 50 mg/m^2 of oral etoposide for 21 days every 28–35-day cycles. Partial responses were achieved in 60 % patients (95 % CI 41–77 %). Median time to disease progression was 5 months (range 2–13 months) overall, while median time to progression was 8 months in indolent lymphoma compared with only 3 months in aggressive lymphoma [59].

12.3.2.3 Vinblastine

In a prospective study of 36 pediatric patients, weekly treatment with single-agent vinblastine at 6 mg/m^2 has been shown to be effective for relapsed or refractory anaplastic large cell lymphoma (ALCL) [60]. In 30 evaluable patients, the CR rate was 83 %, and the responses appeared to be durable. In 25 patients who achieved CR, 9 remained in CR after a median follow-up of 7 years. While the optimal duration of therapy remained to be defined, the median duration of treatment was 30 months in patients who had long-lasting CR.

12.3.2.4 Cyclophosphamide-Based Regimens

Buckstein and colleagues reported the clinical efficacy of oral combination of low-dose oral cyclophosphamide and Cox-2 inhibitor celecoxib, which have been shown to suppress tumor angiogenesis, in relapsed aggressive non-Hodgkin lymphoma [61]. Patients were treated with oral cyclophosphamide 50 mg daily and high-dose celecoxib at 400 mg twice daily. In 35 heavily pretreated DLBCL patients, the overall best response rate was 37 %, with 22 % achieving stable disease. These observed clinical responses appeared to correlate with declining levels of circulating endothelial cells ($CD45^-CD31^+CD146^+$) and their precursors ($CD45^-CD31^+CD146^+CD133^+$) in responders, suggesting angiogenesis inhibition as a potential mechanism of action.

12.3.2.5 PEPC and Its Derivative

PEPC consists of low-dose prednisone (20 mg), etoposide (50 mg), procarbazine (50 mg), and cyclophosphamide (50 mg) administered orally with dosing frequency titrated to hematological parameters (i.e., ANC above 1,000). PEPC was investigated in 22 patients with heavily pretreated, recurrent mantle cell lymphoma (MCL). Eighty-two percent achieved an objective response including 46 % complete responses and 36 % partial responses. Responses were durable with median time on therapy at 17 months [62]. Another study analyzed 75 patients with relapsed/refractory lymphoma (26 FL, 14 MZL, 12 SLL, 9 HD, 9 DLBCL, and 5 TCL) who were treated with the PEPC regimen. Sixty-nine percent achieved an objective response (ORR) with 36 % CR and 33 % PR. Patients with indolent histologies appeared to have superior responses and time on therapy relative to those with aggressive histologies [63]. A derivative of the PEPC program, RT-PEPC regimen, which combined PEPC with rituximab and thalidomide to enhance antiangiogenesis effects, was evaluated in 25 patients with relapsed / refractory MCL. The overall response rate was 73 % (32 % CR and 41 % PR), and the median progression-free survival

was 10 months [64]. Correlative studies demonstrated that plasma VEGF and circulating endothelial cells trended down with treatment, consistent with response to antiangiogenic treatment.

12.3.2.6 Oral Combination for AIDS-Related NHL

An oral regimen consisted of lomustine 50 mg/m^2 on day 1, etoposide 100 mg/m^2 on days 1–3, and cyclophosphamide/procarbazine 50 mg/m^2 each on days 22–26, given every 6 weeks for a total of two cycles, was studied in 49 patients with NHL diagnosed in setting of HIV in east Africa [65]. The majority of patients were female with poor performance status and advanced stage disease. 18 patients (37 %) had access to antiretroviral therapy. The ORR was 78 %. Median follow-up time was 8.2 months, median EFS was 7.9 months, and median OS was 12.3 months. Thirty-three percent of patients survived for 5 years.

12.3.3 Multiple Myeloma

12.3.3.1 Regimens with Continuous Oral Cyclophosphamide

Continuous administration of oral cyclophosphamide at 50 mg per day given with prednisone at 15 mg per day was retrospectively evaluated in 27 patients with relapsed and refractory MM. The ORR for the CP regimen was 67 %. The median time to response was 2 months, with median PFS not reached in responders at the time of publication [66].

The Hoosier Oncology Group conducted a phase II trial of oral cyclophosphamide (50 mg b.i.d. for 21 days), in combination with thalidomide (200 mg/day) and prednisolone (50 mg q.o.d), given every 28-day cycle, in 37 patients with relapsed MM [67]. The reported ORR of the CTP regimen was 63 % in the evaluable 35 patients, with 20 % (7) CR, 6 % (2) near CR, and 37 % (13) PR. The median time to best response and time to progression were 3.6 and 13.2 months, respectively. The median number of treatment was seven cycles (range 1–12 cycles). Grade 3–4 toxicities were expected and included 43 % leukopenia, 11 % febrile neutropenia, 11 % sensory neuropathy, 6 % motor neuropathy, and 8 % thrombosis.

The continuous low-dose oral cyclophosphamide (50 mg/day) was also studied in combination with bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11 for cycles 1–8 and then 1.3 mg/m^2 on days 1, 8, 15, and 22 for cycles 9–11) and dexamethasone (20 mg/day on the day of bortezomib and the day thereafter) in a phase II trial for patients with relapsed advanced MM [68]. The treatment consisted of eight 3-week cycles, followed by three 5-week cycles. In 50 evaluable patients, complete, partial, and minor responses were recorded at 16, 66, and 8 %, respectively, with ORR at 90 %. Median event-free survival was 12 months, with a median overall survival of 22 months. A/Es were typical and included cytopenias, infection, and neuropathy. Antiviral prophylaxis was mandatory.

12.3.3.2 Regimens with Weekly Oral Cyclophosphamide

Weekly cyclophosphamide has been the backbone of effective outpatient myeloma therapy. In a phase II study in the UK with 52 patients with relapsed or refractory

MM, oral weekly cyclophosphamide at 300 mg/m^2 was combined with pulsed dexamethasone at 40 mg/day for 4 days per month and once daily thalidomide at escalating doses based on tolerance to a maximum of 300 mg [69]. At a median follow-up of 18 months, 17 % patients achieved CR, 62 % PR, and 11 % MR, resulting in ORR of 90 %. The estimated 2-year OS and EFS were 73 and 34 %, respectively, and median TTP was not reached. The CDT regimen appeared to be safe, well tolerated, and effective in the relapsed setting.

Weekly cyclophosphamide (300 mg/m^2) was also studied in combination with bortezomib and pulse steroid in both relapsed and frontline settings. In relapsed setting, bortezomib was provided up to 1.3 mg/m^2 on days 1, 4, 8, and 11, or up to 1.5 mg/m^2 weekly on days 1, 8, and 15, every 28-day cycle. Prednisone was given at 100 mg on alternate days. The ORR was 95 %, with CR in more than 50 % patients at the maximal dose combination in this phase I–II study. The weekly bortezomib combination had less cytopenias and neuropathy. The 1-year PFS and OS were 83 and 100 %, respectively [70]. In previously untreated patients, bortezomib was provided at 1.3 mg/m^2 on days 1, 4, 8, and 11, with oral dexamethasone pulses on days 1–4, 9–12, and 17–20 on a 28-day cycle for four cycles. The CyBorD regimen produced a rapid response with manageable toxicities. The ORR was 88 % on an intent-to-treat basis, with 61 % very good partial response, and 39 % CR or near CR [71].

12.4 Discussion

In summary, metronomic chemotherapy represents a novel and promising strategy, which targets tumor angiogenesis. Preclinical data and biomarker studies have indicated that metronomic chemotherapy interacts with different cells within the tumor microenvironment, including endothelial cells, pericytes, CEPs, and tumor-infiltrating immune cells, contributing to its antiangiogenic and immunomodulatory antitumor effects. Growing clinical data in patients with hematological malignancies have demonstrated clinical benefits including durable objective responses in selected patient populations.

Metronomic chemotherapy is convenient and generally well tolerated. Its clinical applications have been particularly appealing in a number of clinical scenarios, including patients with refractory disease to multiple chemotherapy regimens, patients with significant comorbidities who are not candidates for conventional MTD type of treatment, and patients in developing countries who may not have adequate access to standard induction chemotherapy.

Another unique feature of metronomic chemotherapy is the multitude of clinical feasibilities of combining low-dose chemotherapy with many of the new targeted and biological compounds to augment antitumor effects, thus improving clinical response without added toxicity. (1) One such possibility is to combine metronomic chemotherapy with specific antiangiogenic tyrosine kinase inhibitors or vascular disrupting agents to enhance antiangiogenesis. (2) Another promising clinical development involves combining metronomic chemotherapy backbone with biologics specific for the tumor cells. An example of this application is the combination of

anti-myeloma-specific proteasome inhibitor bortezomib with low-dose cyclophosphamide for myeloma patients. Combining specific anti-B-cell agents including monoclonal antibodies and B-cell kinase inhibitors with low-dose metronomic therapy appears worth exploring for patients with B-cell non-Hodgkin lymphoma as well. (3) Finally, harnessing its immunomodulatory antitumor effect, metronomic therapy has shown therapeutic benefit when combined with immunomodulatory compound thalidomide in patients with myeloma and lymphoma. Current clinical trials are ongoing to assess the safety and efficacy of the combination of lenalidomide with low-dose melphalan to treat high-risk MDS (NCT00744536) and the combination of lenalidomide with low-dose treosulfan for patients with multiple myeloma (NCT01010243).

Further understanding of the disease-specific and drug-specific mechanisms underlying metronomic therapy will provide critical insights for the rational design of future effective metronomic combination and schedules that are tailored to disease subtypes. Prospective correlative studies should be included with clinical trials whenever possible to validate mechanisms of action and clinically useful prognostic biomarkers. Lastly, optimal duration of metronomic therapy needs to be better defined in prospective studies with close monitoring to minimize potential long-term toxicities, including bone marrow dysplasia and secondary malignancies, which can be associated with prolonged exposure to chemotherapeutic agents.

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Clinical Activity of Metronomic Chemotherapy in Liver Cancers

13

Yu-Yun Shao, Ann-Lii Cheng, and Chih-Hung Hsu

Abstract

Treating advanced hepatocellular carcinoma (HCC) remains challenging in clinical practice. Although sorafenib, an antiangiogenic targeted compound, has demonstrated survival benefits as a first-line therapy, the response rate and time to progression are not optimal. Metronomic chemotherapy has demonstrated antiangiogenic effects, and its reduced potential for toxicity renders it more tolerable to most advanced HCC patients. Clinical trials and retrospective studies have examined the use of metronomic chemotherapy, either alone or in combination with other antiangiogenic therapies, for treating advanced HCC. These studies have confirmed the feasibility and safety of metronomic therapy in patients with advanced HCC. Although objective responses were achieved using metronomic chemotherapy alone, it is difficult to discern the actual clinical benefits because of the small sample sizes of these studies. Nevertheless, metronomic chemotherapy can serve as a treatment option for advanced HCC patients who have progressed on or are intolerable to the standard therapy, sorafenib. In single-arm phase II clinical trials, combining metronomic chemotherapy with antiangiogenic targeted therapy has demonstrated improved efficacy for treating advanced HCC without increasing toxicities. Further research is warranted to confirm the benefits of combining metronomic chemotherapy with antiangiogenic targeted therapy for treating advanced HCC.

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13.1 Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the second leading cause of cancer-related mortality worldwide [1]. In areas with a high prevalence of viral hepatitis, HCC has become the leading cause of cancer-related deaths in recent decades. Localized HCC can be cured by resection, local ablation therapies, or liver transplantation [2]. Unfortunately, the majority of localized HCCs develop recurrent or metastatic disease that is no longer treatable by locoregional therapies.

Advanced HCC is defined as locally advanced or metastatic HCC that is no longer amenable to locoregional therapies. Systemic therapy is indicated for advanced HCC. However, cytotoxic chemotherapy, which has been the major type of cancer therapy used in previous years, has been shown to be ineffective for advanced HCC [3–6]. Furthermore, most advanced HCC patients are not amenable to conventional cytotoxic chemotherapy because of cytopenia and diminished liver function caused by chronic hepatitis and cirrhosis.

Such a disappointing condition has changed since 2008. Two large randomized phase III trials have demonstrated that sorafenib, compared to a placebo, improved overall survival in patients with advanced HCC [7, 8]. Sorafenib is a multi-kinase inhibitor that targets the vascular endothelial growth factor (VEGF) receptor and Raf kinase [9], through which it inhibits angiogenesis and cancer cell proliferation. Because sorafenib has clearly demonstrated clinical benefits in treating advanced HCC, it became the first ever and remained the only therapeutic agent approved for the treatment of advanced HCC.

13.2 Challenges in Treating Advanced HCC

However, the efficacy of sorafenib in advanced HCC is modest [7, 8]. The objective tumor response rate of sorafenib is 2–3 %, and the disease stabilization rates range from 34 to 43 % [7, 8]. In a randomized phase III trial conducted in East Asia, the median time to progression for patients with advanced HCC treated with sorafenib as a first-line therapy was only 2.8 months [7]. Thus, improving the efficacy of sorafenib and developing novel therapeutics are crucial for improving systemic therapy for advanced HCC.

However, despite continued efforts, none of the new multi-kinase inhibitors or new classes of targeted therapy have shown improved efficacy in treating advanced HCC. In large-scale randomized phase III trials, sunitinib, brivanib, and linifanib have failed to demonstrate greater clinical benefits as a first-line treatment for advanced HCC than sorafenib has [10–12]. In patients for whom sorafenib had failed, brivanib and everolimus have also failed to show significant survival benefits [13, 14]. Recently, large-scale next generation sequencing analyses of HCC cells were performed [15–18]. However, although a more comprehensive view of the genetic alterations that occur in HCC has begun to emerge, these findings have not yet led to the development of new therapeutic strategies for HCC.

13.3 Scientific Basis and Advantages of Metronomic Chemotherapy for Advanced HCC

Antiangiogenic therapy is considered vital for treating advanced HCC, which is most often characterized by hypervascularity. The imaging findings regarding vascularity are included in the clinical diagnostic criteria of HCC [19]. The only approved treatment for advanced HCC, sorafenib, produces antiangiogenic effects by blocking the VEGF receptor. Other antiangiogenic compounds, such as bevacizumab, sunitinib, and brivanib, have also demonstrated some efficacy in advanced HCC [20–25].

Metronomic chemotherapy refers to administering chemotherapeutics at doses significantly less than the maximum-tolerated dose (MTD), on frequent dosing intervals, for a prolonged period [26]. Preclinical models have demonstrated the antiangiogenic activity of metronomic chemotherapy [27, 28]. In animal studies, metronomic chemotherapy can suppress tumor growth, inhibit distant metastasis, prolong survival, and diminish tumor angiogenesis [29–31]. Combining antiangiogenic targeted therapy with metronomic chemotherapy may improve outcome further. Animal studies have demonstrated that combination therapy inhibited tumor growth, prolonged survival, and delayed resistance to antiangiogenic therapy [32–35]. Thus, metronomic chemotherapy, either alone or in combination with antiangiogenic targeted therapy, may be considered a treatment option for advanced HCC.

A potential advantage of metronomic chemotherapy is minimal bone marrow toxicity, which improves tolerance among patients. Patients with advanced HCC often have cirrhosis of the liver caused by chronic liver disease, which frequently results in cytopenia and impaired organ function. Patients with advanced HCC are usually poor candidates for MTD-type cytotoxic chemotherapy.

13.4 Clinical Trials of Metronomic Chemotherapy for Advanced HCC

Because of its antiangiogenic effects and favorable toxicity profile, metronomic chemotherapy has been considered an option for treating advanced HCC. In recent years, a few clinical trials and retrospective studies have examined the efficacy of metronomic chemotherapy, either alone or in combination with other antiangiogenic therapy, in patients with advanced HCC. The regimens of metronomic chemotherapy used in these studies have consisted primarily of oral fluoropyrimidine derivatives, such as capecitabine or tegafur/uracil. We discuss these studies herein.

13.4.1 Metronomic Chemotherapy Alone

Two prospective clinical trials of the use of metronomic chemotherapy alone for treating advanced HCC have been reported (Table 13.1). The study by Brandi et al. [37] evaluated the efficacy of capecitabine. Capecitabine is an oral prodrug of

Table 13.1 Clinical studies of metronomic chemotherapy with oral fluoropyrimidines alone for hepatocellular carcinoma

Authors	Regimens	*N*	Patients	Prior systemic therapy	Response rate (%)	Disease control rate (%)	Median PFS (months)	Median OS (months)
Clinical trials								
Ishikawa et al. [36]	Supportive care only Tegafur/uracil 400 mg bid (based on tegafur), continually	20 28	Locally advanced HCC[a]	Nil	– 18	– –	– –	6.2 12.1
Brandi et al. [37]	Capecitabine, 500 mg bid, continually Capecitabine, 500 mg bid, continually	59 31	Advanced HCC Advanced HCC	Nil Sorafenib	5 0	56 32	6.0 3.3	14.5 9.8
Retrospective studies/case reports								
Ishikawa et al. [38]	Tegafur/uracil 400 mg/day (based on tegafur), continually	1	Advanced HCC HCV (+)	Nil	Complete response; PFS/OS = 18 months; death due to rectal ulcer bleeding			
Ballardini et al. [39]	Capecitabine, 500 mg bid, continually	1	Advanced HCC HBV (+)	Nil	Partial response; PFS/OS >18 months			

Abbreviations: *PFS* progression-free survival, *OS* overall survival, *HCC* hepatocellular carcinoma, *HBV* hepatitis B virus, *HCV* hepatitis C virus

[a]Include multiple tumors in more than one lobe, a tumor or tumors involving a major branch of the portal or hepatic veins, or lymph node metastasis

5-fluorouracil and is commonly used to treat colorectal, gastric, and breast cancers [40–43]. The study enrolled two cohorts of patients with advanced HCC to be treated with capecitabine (500 mg) twice daily continually [37]. The first cohort consisted of 59 previously untreated patients with advanced HCC. The response rate (RR) was 5 %, and the disease control rate (DCR) was 56 %. The median progression-free survival (PFS) was 6.0 months, and the median overall survival (OS) was 14.5 months. The second cohort consisted of 31 patients who were resistant or intolerant to sorafenib. No response was identified, but 32 % of the patients experienced disease stabilization. The median PFS was 3.3 months, and the median OS was 9.8 months. The disease control rate was comparable with that reported in two phase II trials that have used brivanib (46 %) and tivantinib (42 %) as a second-line treatment for advanced HCC [22, 23].

Another prospective study evaluated metronomic chemotherapy using tegafur/uracil for advanced HCC. Tegafur/uracil is an oral fluoropyrimidine that is approved for and commonly used in treating gastric, colorectal, and non-small cell lung cancers in patients in Asian countries [44–46] (Table 13.1). Ishikawa et al. [36] randomized 48 patients with locally advanced HCC to receive supportive care only or to continually receive 400 mg of tegafur/uracil (based on tegafur) twice daily. Among the 28 patients who received tegafur/uracil, the RR was 18 %, and the median OS was 12.1 months. By contrast, the median OS of patients receiving supportive care was 6.2 months.

Overall, these phase II metronomic chemotherapy studies have demonstrated the safety and feasibility of treating advanced HCC patients with metronomic oral fluoropyrimidines. They have demonstrated that either capecitabine or tegafur/uracil could induce objective tumor response in treatment-naïve patients with advanced HCC. For patients who were resistant or intolerant to sorafenib, one study demonstrated that metronomic capecitabine induced disease stabilization in 32 % of advanced HCC patients. These data and those from case reports, which demonstrated significant tumor response for a prolonged period in HCC patients treated with metronomic chemotherapy using either capecitabine or tegafur/uracil [38, 39], collectively indicate that metronomic chemotherapy with oral fluoropyrimidines is clinically effective in certain HCC patients. However, because each of these studies has relatively few patients, caution must be exercised when interpreting the results.

13.4.2 Metronomic-Like Use of Oral Fluoropyrimidine Chemotherapy

Additional clinical studies have evaluated oral fluoropyrimidines by using schedules that deviated from a typical metronomic schedule [26], such as treatment for multiple weeks followed by 1 week of no treatment. The results of these studies on the metronomic-like use of oral fluoropyrimidines in advanced HCC patients are summarized in Table 13.2.

Three studies have evaluated the use of capecitabine (1,000 mg/m^2) twice daily from day 1 to day 14 (2 weeks “on”), followed by no treatment from day 15 to day

Table 13.2 Clinical studies of oral fluoropyrimidine chemotherapy in a metronomic-like schedule for hepatocellular carcinoma

Authors	Regimens	*N*	Patients	Prior systemic therapy	Response rate (%)	Disease control rate (%)	Median PFS (months)	Median OS (months)
Clinical trials								
Abdel-Rahman et al. [47]	Capecitabine, 1,000 mg/m^2 bid, D1–14, every 21 days Sorafenib 400 mg bid	26 26	Advanced HCC	Nil	3 15	58 77	4.0 6.0	5.1 7.1
Retrospective studies/case reports								
Mani et al. [48]	Tegafur/uracil 300 mg/m^2/day and leucovorin 90 mg/day D1–28, every 35 days	14	Advanced HCC HBV (+) 0 %; HCV (+) 29 %	Nil	0	21	4.5[a]	> 10
Patt et al. [49]	Capecitabine, 1,000 mg/m^2 bid, D1–14, every 21 days	37	Advanced HCC HBV (+) 19 %; HCV (+) 32 %	Yes 41 % No 59 %	11	22	–	10.1
von Delius et al. [50]	Capecitabine, 1,000 mg/m^2 bid, D1–14, every 21 days	11	Advanced HCC HBV (+) 9 %; HCV (+) 18 %	Yes 27 % No 73 %	9	27	2.2	10.1
Di Meglio et al. [51]	Tegafur/uracil 500 mg/day (based on tegafur), D1–28, every 35 days	1	Advanced HCC HCV (+)	Nil	Partial response; PFS ~ 32 months; OS > 44 months			

Abbreviations: *PFS* progression-free survival, *OS* overall survival, *HCC* hepatocellular carcinoma, *HBV* hepatitis B virus, *HCV* hepatitis C virus
[a]Time to progression reported in the study

21 (1 week "off") [47, 49, 50]. Patt et al. [49] retrospectively analyzed 37 patients with HCC who were not amenable to locoregional therapies and found that capecitabine treatment induced a partial response rate of 11 % and a disease stabilization rate of 22 %. In a retrospective analysis of 11 patients with advanced HCC, von Delius et al. [50] reported that one patient experienced partial response (9 %) lasting 13 months and two other patients experienced disease stabilization.

The only prospective study was conducted by Abdel-Rahman et al. [47], who enrolled 52 patients with advanced HCC and randomized them to receive either capecitabine or sorafenib as a first-line therapy. Among the 26 patients who received capecitabine, the RR was 3 %, and the median PFS and OS were 4.0 and 5.1 months, respectively. Compared with patients treated using sorafenib, those treated using capecitabine had poorer outcomes, including poorer RR and shorter PFS and OS.

Two trials have evaluated the efficacy of tegafur/uracil by using a 28-day-on, 7-day-off schedule in patients with advanced HCC (Table 13.2). In a phase II study, Mani et al. [48] enrolled 16 advanced HCC patients and treated them with tegafur/uracil (300 mg/m^2/day) and leucovorin (90 mg/day) from day 1 to day 28, followed by no treatment for 1 week, repeated every 35 days. Fourteen patients were evaluable for response. Although no patients experienced objective responses, disease stabilization occurred in three patients for 17–22 weeks. The median time to progression and overall survival time were 4.5 and >10 months, respectively. In another case report, an HCC patient treated using a repeated schedule of tegafur/uracil (500 mg/day) from day 1 to day 28, followed by no treatment for 1 week, showed a partial response [51].

These data collectively suggest that the use of oral fluoropyrimidines in advanced HCC patients following a metronomic-like schedule provides a safe toxicity profile and modest clinical activity (Table 13.2) that are similar to those of the metronomic use of oral fluoropyrimidines in patients with advanced HCC (Table 13.1). However, interpreting the findings of these studies is limited by the retrospective nature of the study design and/or the relatively small sample size. No studies have addressed the dosages and/or schedules of oral fluoropyrimidines regarding antitumor activity in advanced HCC.

13.4.3 Antiangiogenic Therapy Combined with Metronomic Chemotherapy

Preclinical models have demonstrated the synergistic antitumor activity of metronomic chemotherapy combined with other antiangiogenic drugs [28, 32–35]. Three single-arm phase II trials and a retrospective study evaluated various combinations as first-line therapies for patients with advanced HCC in Asian countries (Table 13.3).

Hsu et al. [52] examined the efficacy and safety of combining metronomic tegafur/uracil with sorafenib as a first-line therapy for patients with advanced HCC. Fifty-three patients with Child-Pugh class A liver reserve and adequate organ functions were enrolled to receive continual sorafenib (400 mg) twice daily and tegafur/uracil (125 mg/m^2 based on tegafur) twice daily. The RR was 8 %, and the DCR was 57 %.

Table 13.3 Clinical studies of metronomic chemotherapy combined with antiangiogenic therapy for hepatocellular carcinoma

Authors	Regimens	*N*	Patients	Prior systemic therapy	Response rate (%)	Disease control rate (%)	Median PFS (months)	Median OS (months)
Clinical trials								
Hsu et al. [52]	Sorafenib, 400 mg, bid Tegafur/uracil, 125 mg/m^2 (based on tegafur), bid Both continually	53	Advanced HCC HBV (+) 72 %; HCV (+) 25 %	Nil	8	57	3.7	7.4
Hsu et al. [53]	Bevacizumab, 7.5 mg/kg, day one, triweekly Capecitabine, 800 mg/m^2, bid, D1 to 14, every 21 days	45	Advanced HCC HBV (+) 67 %; HCV (+) 18 %	Nil	9	52	2.7	5.9
Shao et al. [54]	Thalidomide, 200 mg/day Tegafur/uracil, 125 mg/m^2 (based on tegafur), bid Both continually	43	Advanced HCC HBV (+) 72 %; HCV (+) 14 %	Nil	9	33	1.9	4.6
Retrospective studies/case series								
Ang et al. [55]	Thalidomide 50–200 mg/day Capecitabine, 1,000 mg/m^2 bid, D1–14, every 21 days	42	Advanced HCC HBV (+) 60 %; HCV (+) 7 %	Yes (17 %)	14 (CR 7)	45	5.1	9.9

Abbreviations: *PFS* progression-free survival, *OS* overall survival, *HCC* hepatocellular carcinoma, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *CR* complete response

The median PFS was 3.7 months, and the median OS was 7.4 months. The treatment was well tolerated, and the grade 3 or 4 adverse events, including fatigue (15 %), hand-foot skin reaction (9 %), and bleeding (8 %), were relatively infrequent. Compared with other reports using sorafenib alone in treating patients with advanced HCC, the combination therapy demonstrated no increased toxicity.

The second study examined the feasibility and efficacy of using bevacizumab combined with capecitabine as a first-line therapy for advanced HCC [53]. Bevacizumab is a monoclonal antibody that binds the VEGF. It has been approved for treating various malignant diseases, including advanced colorectal and non-small cell lung cancers [56, 57]. Bevacizumab (7.5 mg/kg) was administered intravenously every 3 weeks. Capecitabine (800 mg/m^2) was administered orally twice daily from day 1 to day 14, followed by no treatment for 1 week, and the treatment was repeated every 3 weeks. A total of 45 patients were enrolled. The objective RR was 9 %, and the DCR was 52 %. The median PFS was 2.7 months, and the median OS was 5.9 months. Treatment was well tolerated, with no grade 3 or 4 hematological toxicities. The most frequent grade 3 or 4 adverse reactions were gastrointestinal bleeding (9 %), hand-foot skin reaction (9 %), and diarrhea (4 %).

The third trial evaluated the use of thalidomide combined with tegafur/uracil as a first-line therapy in 43 advanced HCC patients [54]. Thalidomide has been shown to provide clinical benefits to patients with advanced HCC in single-arm phase II trials, which have reported RRs ranging from 3 to 7 % [58–61]. The antitumor activity of thalidomide is partially attributable to its antiangiogenic properties and has been shown to inhibit both basic fibroblast growth factor- and VEGF-induced angiogenesis in corneal micropocket assays [62, 63]. In this study, patients were treated with thalidomide (200 mg/day) and tegafur/uracil (125 mg/m^2 based on tegafur, bid) continuously [54]. An RR of 9 % and a DCR of 33 % have been reported. The median PFS was 1.9 months, and the median OS was 4.6 months. The treatment was well tolerated, with infrequent grade 3 or 4 adverse events. The most common grade 3 or 4 treatment-related adverse events were somnolence (9 %), gastrointestinal hemorrhage (5 %), skin rashes (2 %), and dizziness (2 %).

These three phase II trials have collectively demonstrated the feasibility of combining antiangiogenic therapy with metronomic oral fluoropyrimidine chemotherapy and have shown encouraging clinical efficacy in advanced HCC, with objective RRs of 6–9 % and DCRs ranging from 33 to 57 %. Another retrospective study of 42 patients who received combination therapy with thalidomide and capecitabine reported an RR of 14 % and a DCR of 45 % [55], corroborating the results of the three aforementioned prospective clinical trials.

Nevertheless, it remains difficult to determine whether combining antiangiogenic targeted therapy with metronomic chemotherapy is more effective against advanced HCC than either metronomic chemotherapy or antiangiogenic therapy alone. No randomized studies have directly compared a combination approach with a single treatment modality. Furthermore, a cross-study comparison of various clinical trials of advanced HCC is problematic because of the heterogeneous disease states and variable outcomes among different studies.

Prior studies have shown that patients with advanced HCC may have variable outcomes [64, 65]. Studies on patients from different geographic areas or on patients with various etiological factors may yield distinct outcomes, despite having similarly advanced diseases. In two pivotal studies of sorafenib treatment for advanced HCC, the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) study enrolled patients from Europe, Australia, and New Zealand [8], and the Asia-Pacific study enrolled patients from China, South Korea, and Taiwan [7]. Despite using the same inclusion/exclusion criteria for patient selection and the same dose and schedule of sorafenib, the efficacy outcomes of the two studies differed significantly. Compared with the patients in the Asia-Pacific study, the patients in the SHARP study had longer time to progression (5.5 vs. 2.8 months) and OS (10.7 vs. 6.5 months).

Conclusion

A limited number of clinical trials have thus far been conducted to evaluate the efficacy of metronomic chemotherapy for advanced HCC. The clinical trials that have been performed primarily used oral fluoropyrimidines. The findings of these trials confirm that metronomic chemotherapy is well tolerated by advanced HCC patients and may have modest antitumor activity for advanced HCC. Metronomic chemotherapy using oral fluoropyrimidines can thus serve as a treatment option for advanced HCC patients who have progressed on or cannot tolerate sorafenib, which is the current standard for first-line treatment.

Combining antiangiogenic therapy with metronomic chemotherapy, the sound scientific basis of which has been shown in preclinical models, has been evaluated in three prospective phase II studies. The results indicate that the combination treatment does not increase the potential for toxicity and is well tolerated by advanced HCC patients. The encouraging antitumor activity demonstrated in these phase II studies warrants future investigation to confirm the clinical benefits of combining antiangiogenic therapy with metronomic chemotherapy for treating advanced HCC patients.

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Metronomic Chemotherapy in Gynecological Cancers

14

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Abstract

Ovarian cancer is the 5th leading cause of cancer mortality in the United States, and despite major advances in cytoreductive surgery and the use of chemotherapy with a platinum and a taxane in the first-line setting, recurrence is still a common problem. Efforts to improve the efficacy of conventional chemotherapy have been resulted in general in limited benefit. Metronomic chemotherapy represents an alternate schedule of chemotherapy administration. Preclinical and clinical data attest to the efficacy of metronomic chemotherapy as a treatment modality in ovarian cancer. Further research, including phase III clinical studies, is required to determine the role of this promising therapeutic approach in the management of ovarian cancer.

14.1 Introduction

Ovarian, endometrial, and cervical cancers represent the most common gynecological tumors. Of these, ovarian cancer is the most lethal. It is estimated that in 2013 in the United States 22,240 women will be diagnosed with and 14,030 women will die of ovarian cancer, making ovarian cancer the fifth leading cause of cancer death [1]. While advances in cytoreductive surgery and the use of first-line chemotherapy with platinum and taxane have increased disease-free survival and overall survival [OS], recurrence is still a common problem [2]. Most patients present with advanced disease [stage III–IV], and only 25–30 % of them are alive at 5 years [1, 2]. Treatment for recurrent platinum-sensitive disease can achieve long-term control [3, 4]. However, all patients with recurrent disease will eventually develop resistance to platinum. In this setting, several agents, such as pegylated liposomal doxorubicin, topotecan, taxanes, etoposide, and gemcitabine, have activity [2]. However, response rates to single agent are only 10–25 %, and median survival is less than 1.5 years [5].

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Combination chemotherapy is frequently associated with a higher response rate and increased toxicity, but this has not translated into improved survival [6–8].

Metronomic chemotherapy represents an alternative to using more intense, toxic chemotherapy regimens in patients with recurrent ovarian cancer. The mechanisms of action and preclinical studies of metronomic chemotherapy are presented in detail in Chaps. 2 and 3 of this book. Briefly, standard chemotherapeutic regimens are designed to deliver the highest or maximum tolerated dose [MTD], which can be safely administered [9]. Due to detrimental effects on normal tissues, rest periods of typically 3–4 weeks are required between treatments and to minimize toxicity. However, recent studies indicate that tumor-associated endothelial cells continue to proliferate and promote cancer growth between treatments [10, 11].

Therefore, the "more is better" philosophy may not be ideal. To avoid the toxicities and morbidity caused by conventional chemotherapeutic regimens and improve the quality of life of cancer patients, several groups had studied a new modality of drug administration: metronomic chemotherapy [12]. This term was first used by Douglas Hanahan, who also emphasized the concept of "less is more" and demonstrated the antiangiogenic effect of metronomic dosing of cytotoxic agents in mice [13].

Similar definitions include the administration of cytotoxic drugs on a more continuous basis, with a much shorter break period, or none at all, and generally at lower doses of various cytotoxic drugs or combinations with other newer, targeted therapies, like antiangiogenesis agents [14].

Metronomic chemotherapy is associated with lower treatment-related toxicity than conventional maximum tolerated dose (MTD) chemotherapy, and phase II/III trials are revealing that it is active [15].

It has been proposed that metronomic chemotherapy exerts its antitumoral effects primarily by inhibiting angiogenesis and regulating immune response [11, 16]. In preclinical models, virtually every class of chemotherapeutic agent administered on a metronomic schedule has been shown to inhibit angiogenesis, which contributes to their antitumor efficacy [17]. Furthermore, impressive antiangiogenic and antitumor effects and reduced toxicity have been observed in mice [18]. In the rest of this chapter, we will discuss the experimental and clinical data that has investigated the efficacy and toxicity of metronomic chemotherapy in gynecological tumors. As virtually all research has been conducted in ovarian cancer, we will limit our discussion to this disease.

14.2 Metronomic Chemotherapy in Ovarian Cancer

The management of ovarian cancer begins with appropriate surgical staging and tumor debulking followed by platinum-based chemotherapy. The administration of 6 cycles of intravenous carboplatin and paclitaxel represents the standard treatment for patients with stage III–IV [2]. However, in early disease the optimal number of cycles has not been determined [19]. Over the last 20 years, several strategies have

been evaluated to improve the outcome of ovarian cancer. Some, such as the addition of a third cytotoxic chemotherapy agent, have been completely unsuccessful [20]. Others have clearly shown an improved outcome in randomized trials. For example, the administration of intraperitoneal chemotherapy [IP] has consistently shown an improvement in survival in patients with optimally debulked ovarian cancer [21–23]. However, for various reasons, this approach is not widely used [24]. Recent studies suggest that the use of paclitaxel on a dose-dense schedule improves survival [25]. This approach awaits confirmation from other studies. The addition of bevacizumab was shown to have modest effects [26, 27].

Another approach that was studied to improve the outcome of ovarian cancer was to administer paclitaxel as a maintenance treatment after completing standard chemotherapy with carboplatin and paclitaxel. In protocol SWOG 9761/GOG 178, patients with stage III ovarian cancer who had no evidence of disease after completing 6 cycles of standard treatment were randomized to receive 3 or 12 additional cycles of paclitaxel at a dose of 175 mg/m^2 [28]. In GOG 175, patients with stage I–II disease were randomized to maintenance treatment with 24 weeks of low-dose paclitaxel or observation after 3 cycles of intravenous carboplatin and paclitaxel [29]. The results of these trials are discussed later in this chapter and are summarized in Table 14.1.

Patients with recurrent ovarian cancer are categorized into two major groups. Patients who relapse more than 6 months after completing platinum-based chemotherapy are classified as "platinum sensitive," and standard treatment includes retreatment with a platinum-based regimen. Combination regimens appear to be superior to single-agent platinum [3, 4].

Patients who relapse or progress within 6 months of their last platinum-based regimen are considered platinum resistant. All patients with recurrent ovarian cancer will eventually become platinum resistant and are then treated with non-platinum agents such as pegylated liposomal doxorubicin, topotecan, taxane (docetaxel or weekly paclitaxel), gemcitabine, and others. These patients are typically treated with sequential single agents. Although there is limited data, combination cytotoxic chemotherapy is frequently associated with an improved response rate but no improvement in overall survival at the cost of increased toxicity [6–8, 30, 31].

In the next sections, we discuss the experimental and clinical data evaluating the role of metronomic chemotherapy in ovarian cancer, both when used as frontline therapy and in recurrent disease.

14.3 Paclitaxel

The introduction of paclitaxel to frontline treatment leads to a significant improvement of survival in ovarian cancer. Paclitaxel is frequently administered at a maximal tolerated dose every 3 weeks in the initial treatment of ovarian cancer. In vitro data suggest that the duration of exposure plays a crucial role in the cytotoxicity of paclitaxel [32, 33]. Weekly administration of paclitaxel has the potential to have an effect similar to that of continuous infusion while taking advantage of the

Table 14.1 Phase III randomized trials evaluating modifications to carboplatin and paclitaxel as treatment of ovarian cancer

Study	Treatment	Results		Grade 3/4 toxicities (%)		
		PFS	OS			
GOG178/SWOG9761	Paclitaxel 175 mg/m IV q 4 weeks	Median PFS	NR		3 cycles	12 cycles
Stage III	3 cycles	21 m				
Maintenance treatment	12 cycles	28 m		Neutropenia	10	4
$N=277$ (Markman)		HR = 2.31		Neuropathy (grade 2/3)	15	23
		$P=0.023$				
GOG 157	Carboplatin/paclitaxel	5 years PFS	5 years OS		3 cycles	6 cycles
3 vs. 6 cycles	IV q 3 weeks	75 %	81 %	Neutropenia	78	85
		80 %	83 %	Anemia	4.7	6.6
Stage I/II	3 cycles	HR = 0.761	HR = 1.02	Gastrointestinal	4.7	8.9
$N=457$ (Bell)	6 cycles	$P=0.18$	$P=0.94$	Neuropathy	1.8	11.3
GOG 172	IV vs. IP Carboplatin/paclitaxel	Median PFS	Median OS	Leukopenia	IV	IP
				Thrombocytopenia	64	76
IP chemotherapy	IV	18.3 m	49.7 m	Gastrointestinal	4	12
		23.8 m	65.6 m	Renal	24	46
Stage III optimally debulked	IP	HR = 0.77	HR = 0.73	Neuropathy	2	7
				Infection	9	19
$N=429$ (Armstrong)		$P=0.05$	$P=0.03$	Fatigue	6	16
				Metabolic	4	18
				Pain	7	27
					1	11
GOG 218	Carboplatin/paclitaxel IV q 3 weeks × 6	Median PFS	Median OS		Placebo	Bevacizumab
Stage III	Placebo	10.3 m	39.3 m	Thrombocytopenia	15	21
		14.1 m	39.7 m	Hypertension	1.6	10
$N=1,248$ (77)	Bevacizumab 15 mg/kg IV q 3 weeks × 20 cycles	HR = 0.71	HR = 1.03	Constitutional	10	13
		$P=0.001$	$P=0.45$	Gastrointestinal	7.9	19

JGOG 3016	Carboplatin/paclitaxel IV × 6 cycles	Median PFS	Median OS	Neutropenia	Standard	Dose dense
Stage III	Paclitaxel 175 mg/m^2 q 3 weeks	17.5 m	62.2 m	Thrombocytopenia	88	92
		28.2 m	100.5	Anemia	38	44
Dose-dense paclitaxel	Paclitaxel 80 mg/m^2 q 1 week	HR = 0.76	HR = 0.79	Neuropathy	44	69
N = 631 (Katsumata × 2)		*P* = 0.0037	*P* = 0.039		10	12
GOG 175	Carboplatin/paclitaxel IV q 3 weeks × 3	5 years PFS	5 years OS	Neutropenia	Observation	Weekly paclitaxel
Low-dose paclitaxel	Observation	77 %	85.4 %	Cardiovascular	74	75
		80 %	86.2 %	Gastrointestinal	2.6	4.4
Stage I/II	Paclitaxel 40 mg/m^2 weekly × 24	HR = 0.80	HR = 0.78	Infection	4.1	4.5
N = 571 (mannel)		*P* = 0.24	*P* = 0.23	Neuropathy	4.1	5.5
					0.7	4.4

minimal hematological toxicity associated with shorter infusions [34]. Several clinical trials, in ovarian cancer as well as other tumors, have reported that patients who became resistant to this schedule were found to have a high response to paclitaxel administered at a lower dose every week [34–38]. In addition, toxicity, particularly myelosuppression, was decreased.

In summary, clinical trials demonstrated that weekly paclitaxel administered at a dose of 80 mg/m^2 is one of the most active regimens in recurrent platinum-resistant ovarian cancer [35, 36]. In addition, as mentioned above, the substitution of conventional paclitaxel for weekly [or dose-dense] paclitaxel, in combination with carboplatin, was reported to significantly improve progression-free and overall survival in a phase III randomized trial in patients with stage III–IV disease.

The use of an even lower dose of weekly paclitaxel was evaluated in protocol GOG 175 [29]. In this study, patients with stage I or II ovarian cancer were treated with 3 cycles of conventional carboplatin and paclitaxel and were then randomized to observation or 24 weeks of low-dose (40 mg/m^2) paclitaxel. Although it did not achieve statistical significance, the recurrence rate was 19.3 % lower for those randomized to weekly paclitaxel, hazard ratio (HR) 0.807 (95 % CI, 0.565–1.15, $P=0.24$). Similarly, the death rate was 21.9 % lower in the paclitaxel arm (HR 0.781; 95 % CI, 0.522–1.17; $P=0.23$).

The addition of weekly paclitaxel modestly increased toxicity as the incidence of grade 2 or worse peripheral neuropathy (15.5 % vs. 6.0 %), infection or fever (19.9 % vs. 8.7 %), and dermatologic events (70.8 % vs. 52.1 %) was higher ($P<0.001$). There was also a slightly greater incidence of grade 2 or worse cardiovascular events (8.1 % vs. 3.8 %, $P=0.044$) among those on the maintenance regimen. Grade 3 or 4 peripheral neuropathy was reported in 0.7 % of the observation group compared to 4.4 % of the maintenance paclitaxel group ($P=0.012$).

Within the limitation of cross comparison among clinical trials, the results of using low-dose weekly paclitaxel as part of the frontline treatment of ovarian cancer compare favorably with other strategies developed to improve the outcome of ovarian cancer such as IP chemotherapy, maintenance paclitaxel administered at full doses, or additional cycles of carboplatin and paclitaxel (3 vs. 6). Table 14.1 summarizes the HR of these strategies and their toxicities.

It is interesting to observe the clinical application and interpretation of these studies by the medical community. In three large randomized clinical trials and in a meta-analysis [39], IP chemotherapy has been shown to significantly improve overall survival. This led to a clinical alert by the National Cancer Institute (NCI) recommending that IP chemotherapy should be considered in patients with small-volume disease [40]. Treatment guidelines in the United States (National Comprehensive Cancer Network-NCCN and NCI) recommend it use [30]. However, IP chemotherapy is not widely used. On the other hand, evaluating the same data, treatment guidelines by the European Society of Medical Oncology (ESMO) do not fully endorse their use [41]. Similarly, the use of dose-dense weekly paclitaxel is recommended by the NCCN, while ESMO does not consider it a standard of care.

The interpretation of the other studies listed in Table 14.1 is also interesting. Strictly talking, none of the remaining studies (GOG 178, GOG 157, GOG 218, and

GOG 175) met their primary end point as they failed to demonstrate a statistically significant improvement in overall survival. GOG 178 and GOG 218 did demonstrate a statistically significant improvement in PFS that did not translate to an improvement in OS. Despite this, both approaches are included as recommended treatments in the NCCN guidelines, although with level of recommendation grade 2B and 3, respectively. On the other hand, ESMO guidelines do not even address the role of maintenance chemotherapy while they endorse the addition of bevacizumab. GOG 157 did not demonstrate a benefit for administering 6 cycles of chemotherapy to patients with early disease. Nonetheless, NCCN recommends the use of 3–6 cycles of carboplatin and paclitaxel, while ESMO recommends single-agent carboplatin [55, 68].

Of interest, no organization recommends or discusses the use of low-dose weekly paclitaxel, a true metronomic schedule, as used in GOG 175. Granted, this trial failed to demonstrate a statistically significant improvement in PFS or OS. However, the magnitude of the observed benefit (HR of 0.80 and 0.78) and the toxicity profile compare favorably with the findings reported in GOG 178, 157, and 218 which are endorsed by guidelines and/or are commonly used in the community.

14.4 Cyclophosphamide

The antiangiogenic effect of cyclophosphamide was first demonstrated in a murine model of cyclophosphamide-resistant tumors designed to rescue mice by inducing endothelial apoptosis [42]. Clinical activity was reported in solid tumors, such as breast cancer [43].

The potential role for metronomic cyclophosphamide in ovarian cancer was first described by Samaritani who reported the case of a 36-year-old woman with stage IIIC ovarian cancer who failed chemotherapy with paclitaxel and carboplatin as first line and progressed after second line with topotecan. She was placed on low daily dose of cyclophosphamide, and her progression-free survival was 65 months without side effects. She was well during the chemotherapy and lived a normal working and social life [44].

Preclinical data showed an improved outcome for combining metronomic cyclophosphamide with bevacizumab in various tumor models. Based on this, a phase II prospective clinical trial evaluated this combination in recurrent ovarian cancer [45]. Patients with measurable disease and prior treatment with a platinum-containing regimen were eligible. Up to two different regimens for recurrent disease were allowed. Treatment consisted of bevacizumab 10 mg/kg intravenously every 2 weeks and oral cyclophosphamide 50 mg/day. The primary end point was progression-free survival at 6 months. Seventy patients were enrolled. The median number of prior chemotherapy treatments was 2. The probability of being alive and progression-free at 6 months was 56 % (6 % SE). A partial response was achieved in 17 patients (24 %). Median time to progression and survival were 7.2 and 16.9 months, respectively. This data suggested that the combination of metronomic cyclophosphamide and bevacizumab was associated with impressive activity and a very favorable toxicity profile in recurrent ovarian cancer. Subsequent retrospective and prospective studies have confirmed these findings [46–48].

Table 14.2 Phase II clinical trials of bevacizumab alone and metronomic cyclophosphamide alone or in combination in recurrent ovarian cancer

Study	Design	Prior treatment	Grade 3/4 toxicities (%)	Activity
Burger et al. [49]	Phase II single agent *N* = 62	1–2 lines 42 % platinum resistant	Hypertension 11 Thrombosis 3 GI perforation 0 (all grades)	RR = 21 % Estimated 6 month PFS = 40 %
Cannistra et al. [50]	Phase II single agent *N* = 44	2–3 lines 87 % platinum resistant	Hypertension 9 Thrombosis 7 GI perforation 11 (all grades)	RR = 16 % Estimated 6-month PFS = 24 %
Garcia et al. [45]	Phase II bevacizumab plus metronomic cyclophosphamide *N* = 70	1–3 lines 40 % platinum resistant	Hypertension 16 Thrombosis 5 GI perforation 6 (all grades)	RR = 24 % 6 month PFS = 56 %
Kummar et al. [51]	Phase II randomized metronomic cyclophosphamide +/− veliparib *N* = 74	1–4 lines	Grade ≥2 toxicities (%) Lymphopenia 6 Mucositis 1	RR = 13 % 6-month PFS not stated

A limitation of this study is the lack of a control arm. Therefore, the individual contribution of bevacizumab and cyclophosphamide is unknown. The activity of single-agent bevacizumab in recurrent ovarian cancer is well defined, while until recently the activity of single-agent cyclophosphamide was unknown until recently.

Two phase II clinical trials evaluated the activity of single-agent bevacizumab in recurrent ovarian cancer [49, 50] and reported response rates of 21 and 16 % and estimated 6-month PFS of 40 and 24 %, respectively. Recently, the results of a phase II randomized trial of metronomic cyclophosphamide alone or in combination with veliparib were reported in abstract form [51]. Seventy-four patients were enrolled and thirty-six were randomized to cyclophosphamide alone. Median number of prior therapies was 4 [range 1–4]. The response rate to single-agent cyclophosphamide was 13 %. Time to progression or overall survival was not reported. Treatment with oral cyclophosphamide was well tolerated as the only grade 2 or higher toxicities reported were lymphopenia and mucositis observed in 2 and 1 patient respectively.

Table 14.2 summarizes the results of these studies. Within the limitation of cross comparison among trials, the available data suggests that the combination of bevacizumab and metronomic chemotherapy seems to be more active than single-agent bevacizumab or metronomic cyclophosphamide as the response rate is slightly higher, but more importantly the 6-month progression-free survival is among the highest ever reported for recurrent ovarian cancer. These studies also suggest that metronomic cyclophosphamide administered as single agent has a very favorable toxicity profile and activity comparable to that of bevacizumab in this setting.

14.5 Topotecan

Tumor angiogenesis is regulated by a balance of stimulatory and inhibitory factors modulated by both the tumor cells and the tumor microenvironment [52]. Among the stimulatory factors, hypoxia inducible factor [Hif] plays a critical role in hypoxia-mediated angiogenesis [53]. Topotecan, a semisynthetic analogue of camptothecin, is a potent topoisomerase I inhibitor [54] and is currently FDA approved in the United States for the treatment of recurrent ovarian cancer at a dose of 1.5 mg/m^2 daily for 5 days, given as a 30-minute infusion and repeated every 21 days. Along with its cytotoxic effects, topotecan has been suggested to possess potent antiangiogenic properties and is a Hif-1 antagonist [55].

An in vitro and in vivo experiment by Merrit et al. dose-finding and therapy experiments with oral metronomic topotecan was performed in an orthotopic model of advanced ovarian cancer. Tumor vascularity, proliferation, and apoptosis were examined among treatment arms, and in vitro experiments including MTT and Western blot analysis were performed to identify specific antiangiogenic mechanisms of topotecan. The results revealed that compared to controls, metronomic (0.5, 1.0 and 1.5 mg/kg; daily) and maximum tolerated therapy (MTD; 7.5 and 15 mg/kg; weekly) dosing regimens reduced tumor growth in dose-finding experiments, but significant morbidity and mortality were observed with higher doses. Metronomic and MTD topotecan therapy significantly reduced tumor growth in both HeyA8 and SKOV3ip1 models: 41–74 % (metronomic) and 64–86 % (MTD dosing) ($P<0.05$ for both regiments compared to controls). Compared to controls, the greatest reduction in tumor MVD was noted with metronomic dosing (32–33 %; $P<0.01$). Tumor cell proliferation was reduced ($P<0.001$ vs. controls) and apoptosis increased in all treatment arms ($P<0.01$ vs. controls) for both dosing regimens. Endothelial cells demonstrated a significantly higher sensitivity to topotecan using metronomic dosing versus MTD in vitro. Pro-angiogenic regulators Hif-1α and VEGF levels were reduced in vitro [HeyA8 and SKOV3ip1] with topotecan independent of proteasome degradation and topoisomerase I [56].

In addition, Hashimoto et al. developed a preclinical model of advanced human ovarian cancer and tested various low-dose metronomic chemotherapy regimens [57]. Clones of the SKOV-3 human ovarian carcinoma cell line expressing a secretable beta-subunit of human choriogonadotropic (beta-hCG) protein and firefly luciferase were generated and evaluated for growth after orthotopic (i.p.) injection into severe combined immunodeficient mice; a highly aggressive clone, SKOV-3-13, was selected for further study. Mice were treated beginning 10–14 days after injection of cells when evidence of carcinomatosis-like disease in the peritoneum was established as assessed by imaging analysis. Chemotherapy drugs tested for initial experiments included oral cyclophosphamide or topotecan and intraperitoneal irinotecan, topotecan, cisplatin, or paclitaxel given alone or in doublet combinations. In this model, metronomic cyclophosphamide had no antitumor activity, whereas metronomic irinotecan and topotecan had potent activity.

Clinical trials have evaluated the activity of topotecan administered as a protracted low-dose continuous infusion [58–60]. In these studies, topotecan was

administered at a dose of 0.4 mg/m^2/day for 14–21 days. Response rates of 8–35 % were reported, comparable to those achieved with the approved regimen. Neutropenia appears to be significantly lower with continuous infusion, while other toxicities, including anemia and thrombocytopenia, are comparable. However, despite its encouraging activity and favorable toxicity profile, continuous infusion of topotecan is not routinely used probably in part due to the inconveniences and limitations required to administer protracted infusions in daily clinical practice.

With the development of an oral formulation of topotecan, Tillmans et al. performed a phase I trial to determine the MTD of daily oral topotecan [61]. Dose levels of 0.25, 0.50, 0.75, 1.00, and 1.25 mg were studied. Sixteen heavily pretreated patients with various solid tumors were enrolled, with an average of four prior regimens. Mean cycles received on protocol were two (range 1–6). The topotecan Cmax increased linearly with dose, and the median (range) Tmax was 2 h (1–7). The DLT was reached at 1.25 mg (two patients had Gr.3 GI toxicities). Two patients (14 % response) had stable disease (one patient with a minor response and one patient with cervical cancer has stable disease for 7 months on therapy after multiple recurrences on prior regimens). The remaining patients had disease progression. The MTD for phase II evaluation was defined at 1.0 mg daily. The authors concluded that this 28-day cycle was well tolerated at a MTD dose of 1 mg orally daily.

Based on the activity observed with metronomic irinotecan and topotecan, Hashitmoto also evaluated the combination of orally administered metronomic topotecan in combination with pazopanib, a potent tyrosine kinase inhibitor which targets VEGF and platelet-derived growth factor (PDGF) receptors [57]. Pazopanib as a single agent had modest efficacy. However, the high activity of topotecan was significantly enhanced with the addition of pazopanib, with 100 % prolonged survival for the drug combination, after 6 months of continuous therapy. Similarly, findings were reported by Merritt [62].

These findings lead to a phase clinical trial evaluating the combination of metronomic oral topotecan and pazopanib [63, 64]. Twenty-five patients with gynecological tumors were enrolled. The recommended dose for phase I trials was determined to be topotecan 0.25 mg/day with pazopanib 600 mg/day. There were no grade 4 toxicities, and the most common grade 3 toxicities were neutropenia, anemia, and increased transaminases seen in 12, 8, and 8 % of patients, respectively. An overall response rate of 36 % was observed. Twenty-one patients were evaluable for pharmacokinetic studies. No significant drug interactions were observed.

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Metronomic Chemotherapy in Non-Small-Cell Lung Cancer

15

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Abstract

Metronomic chemotherapy is an alternative approach for the administration of systemic chemotherapy that consists of the administration of low doses of a cytotoxic regimen without any interruptions. Endothelial cells of the tumor blood vessels are rapidly proliferating cells that could be affected with the metronomic use of chemotherapy. Indeed, it is strongly believed that this strategy inhibits vascular angiogenesis and subsequently tumor growth mainly through modulating the cancer microenvironment with favorable toxicity profile. In addition, immune suppression is a reality among the vast majority of cancer patients. T regulatory cells (Tregs) are the main representative of suppressive cells, and their increased expression in cancer has been associated with worse prognosis and tumor progression. Low doses of chemotherapeutic agents have been proven capable to restore the normal immune system function through the elimination of immune suppressive cells. Numerous studies have investigated the efficacy of metronomic strategy in NSCLC patients with very promising results. Several chemotherapeutic agents, such as vinorelbine, gemcitabine, cyclophosphamide, and docetaxel, have been tested as monotherapy or in combination with other drugs. Whether other mechanisms, such as tumor dormancy, are also involved in the effectiveness of metronomic administration of chemotherapy or if it is superior to conventional chemotherapy remains questionable, and further investigation is required.

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15.1 Introduction

Non-small-cell lung cancer (NSCLC) remains a lethal disease and the leading cause of cancer-related death in the Western world. Approximately 160,000 deaths occur annually in the United States alone [1]. More than 80 % of patients with lung cancer have NSCLC, and 56 % of all lung cancer patients in the United States are diagnosed with metastatic disease. Patients with metastatic lung cancer have a 5-year survival of 3–4 %. Although important advances have been made in clinical research in oncology and new chemotherapeutic agents are available for the treatment of NSCLC patients, their prognosis remains poor. In these patients, palliative chemotherapy increases overall survival and improves quality of life when compared to supportive care. Patients with advanced disease have a median overall survival (OS) of approximately 10–12 months when treated with combination of two chemotherapeutic agents, and it seems that current therapeutic strategies have reached a plateau of activity in the treatment of NSCLC [2].

Platinum-based doublets are considered the cornerstone treatment for the NSCLC patients. Nevertheless, very often this conventional chemotherapy (CMT) leads to an imbalance between efficacy and tolerance of the induced toxicity. On one hand, CMT is normally administered at doses close to the maximum tolerated dose (MTD), increasing by that way the activity of chemotherapeutic drugs and, thus, facilitating disruption of the DNA of tumor cells, rendering them unable to replicate [2]. On the other hand, the higher the dose, the higher the toxicity that influences the management of the patient and their quality of life. It is obvious that the occurrence of serious adverse events results to dose reduction which, eventually, hampers the efficacy of anticancer treatments. Furthermore, since CMT is administered at the levels of MTD, a period of rest between the cycles of therapy is required, in order to be better tolerated. Hence, this drug-free period may result to tumor regrowth and also favors the growth of selected clones resistant to the therapy, especially if drug-free periods have to be lengthened [2, 3].

An alternative approach to systemic chemotherapy is the metronomic chemotherapy that involves frequent administration of a cytotoxic regimen in a low dose without any interruptions. This approach reflects what Fidler and Ellis said more than 10 year ago, “Cancer is a chronic disease and should be treated like other chronic diseases” [4]. The main characteristics of metronomic chemotherapy are collectively disposed on Table 15.1.

Table 15.1 Main characteristics of metronomic chemotherapy

Dose-dense chemotherapy without any interruptions
Low, minimally toxic doses
No prolonged drug-free breaks
No application of hematopoietic growth factors

15.2 Metronomic Design Targeting Angiogenesis

This new modality of drug administration that is called "metronomic chemotherapy" took its name by Hanahan et al. in 2000. The name is referred to the schedule of administration which consists of chronic, periodical, and low-dose management of chemotherapeutic drugs [5]. The concept of using metronomic chemotherapy was developed by Kerbel et al., and Folkman et al. suggested that chemotherapy might have antitumor efficacy by targeting tumor vasculature [6, 7]. In 2000, Klement et al. and Browder et al. published two preclinical studies, respectively, showing that repeated low doses of chemotherapy have potentially anticancer efficacy in mice [8, 9] (Fig. 15.1).

This novel approach for treating cancer has led to various studies that examined the exact role and efficacy of low-dose chemotherapy. It is strongly believed that this chemotherapy strategy inhibits tumor growth mainly by modulating the cancer microenvironment by disrupting tumor-associated vascular angiogenesis and, at the same time, with limited and manageable toxicity profile. Many of the endothelial cells that compose the wall of tumor blood vessels are immature and constantly proliferating and anticancer agents could affect them [6]. Many preclinical studies have revealed the alternative antitumor effects of metronomic chemotherapy beyond the direct cytotoxic effect that conventional chemotherapy has, suggesting that this approach may have a role in clinical practice [10, 6, 11]. The clinical studies that have been conducted for several types of cancer confirmed the results of preclinical studies [8, 9] that metronomic chemotherapy could offer clinical benefit to patients that were refractory to treatment or relapsed after conventional chemotherapy, although there were cases that this new approach failed to improve patient survival [12, 13].

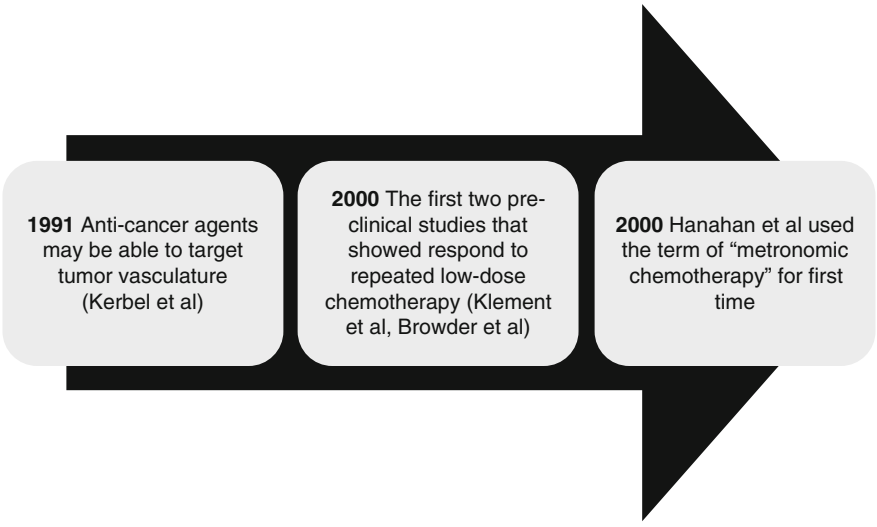

Fig. 15.1 "Metronomic philosophy" development

15.3 Metronomic Design Targeting Immune System

It is known that chemotherapy at dose levels close to MTD has deleterious effect on the immune system by mainly inducing neutropenia and lymphopenia. However, several studies have suggested that certain cytotoxic drugs, including anthracyclines, taxanes, and cyclophosphamide, may exert stimulatory effects on the immune system [14]. In addition, metronomic schedule seems to influence regulatory T cells (T_{REGS}). The frequency of T_{REGS} is increased in distinct tumors, and this event has been correlated with tumor progression and treatment failure [15]. In preclinical models, low doses of cyclophosphamide induce antitumor immune responses by decreasing the expression and inhibiting the suppressive functions of T_{REGS}. Consequently, inhibition of immune suppressive cells leads to the restoration of the normal immunity by increasing both lymphocyte proliferation and memory T cells [16–18]. Recently, it has been shown that metronomic cyclophosphamide led to a decreased number of circulating T_{REGS} and reduced their immunosuppressive functions, in cancer patients with very advanced disease. At the same time, T cell proliferation and NK cell cytotoxic activity were restored in these patients. However, when the same agent was given in higher doses, a depletion of all lymphocyte subpopulations was observed [19]. In a recent in vitro study, various chemotherapeutic agents including vinblastine, paclitaxel, and etoposide were found to able to promote dendritic cell maturation at nontoxic concentrations [20]. These data were confirmed in vivo through the observation that following the administration of vinblastine at low doses, a maturation of tumor-infiltrating dendritic cells was induced [20].

15.4 Metronomic Chemotherapy and NSCLC

Clinical trials investigating the efficacy of metronomic chemotherapy have been conducted in patients with several types of cancer. This therapeutic approach was revealed effective and very well tolerated. In particular, several different agents have been administrated in patients with NSCLC as it is shown in Table 15.2. Since antimitotics are thought to be the most appropriate drugs for metronomic use, because of their capacity to suppress microtubule dynamics and interfere with endothelial cell functionality at very low concentrations [21, 22], numerous clinical studies have tested metronomic administration of vinorelbine and docetaxel with positive results.

Vinorelbine has the advantage of oral formulation, and moreover it serves very well the philosophy of metronomic therapy with a very favorable toxicity profile and significant antitumor activity [30]. Several studies have investigated vinorelbine as an agent that can be used in a metronomic context in patients with NSCLC. In a dose escalation study, metronomic use of oral vinorelbine as monotherapy was tested in patients with recurrent or metastatic breast, prostate, and NSCLC cancer. Thirty-one out of 73 patients who were enrolled in the study had NSCLC. The patients were randomly assigned to 30, 40, or 50 mg of vinorelbine, taken orally

Table 15.2 Major clinical trials with metronomic protocol in NSCLC patients

Study	Metronomic protocol
Pallis et al. (2011) [23]	Vinorelbine 40–70 mg (oral) three times a week plus cisplatin 70–85 mg/m^2 (i.v.) on D1
Briasoulis et al. (2013) [24]	Vinorelbine 50 mg (oral) three times a week
Gorn et al. (2008) [25]	Docetaxel 25 mg/m^2 D1, D8, D15 (i.v.) plus trofosfamide 50 mg daily
Yokoi et al. (2012) [26]	Docetaxel 15 mg/m^2 (i.v.) weekly
Correale et al. (2006) [27]	Cisplatin 30 mg/m^2 D1, D8, D15 (i.v.) plus etoposide 50 mg/m^2 (oral) D1–21
Kakolyris et al. (1998) [28]	Oral etoposide 100 mg/day for seven consecutive days and consequently 100 mg every other day for 14 additional days
Kontopodis et al. (2013) [29]	50 mg p.o. vinorelbine fixed dose three times a week

three times a week. Treatment continued until disease progression, unacceptable toxicity, withdrawal of consent, or maximum 24 months [24]. Two objective partial responses were observed in patients with NSCLC, whereas the side effects of the treatment were mild and negligible. The authors concluded that 50 mg of oral vinorelbine, given three times a week, is the optimal dose for metronomic vinorelbine and suggested further analysis in combination with conventional chemotherapy and/or targeted therapies [24].

Almost the same time, another study with orally given vinorelbine, in heavily pretreated NSCLC patients, was published. Forty-six patients received oral vinorelbine at a fixed dose of 50 mg three times a week until disease progression. Although among the study population 75 % received the treatment as 3rd or further line, the response rate was slightly over 10 % and the disease stabilization reached the levels of 20 % which was considered very encouraging by the authors. Regarding the toxicity, grade 3–4 neutropenia was the most common adverse event observed in 24 % of the patients while febrile neutropenia occurred in 11 % of them. In addition, grade 3 fatigue was the dominant non-hematologic toxicity (11 %) [29].

Docetaxel has also been investigated, as single agent, in a metronomic use. Preclinical studies have revealed that the administration of low-dose metronomic (LDM) docetaxel is pretty feasible and active design for the cancer management. In particular, LDM docetaxel compared to MTD against a human ovarian cancer xenograft model exhibits tumor reduction, leading to improved survival [31]. Metronomic docetaxel has also been shown to decrease the microvessel density of tumors and to inhibit the mobilization of circulating endothelial precursors.

Yokoi et al. conducted another pilot study of metronomic docetaxel as monotherapy [26] with doses of 15 mg/m^2 intravenously, on a weekly basis, without any treatment interruption, until documentation of disease progression. The dose selected was based on a previous phase I study of weekly docetaxel regimen in patients with refractory solid tumors [32]. A total of 27 NSCLC patients were enrolled onto the study who had already been treated with systemic chemotherapy,

thoracic radiotherapy, or surgery. The toxicity profile of the patients was acceptable with no severe hematological adverse events. Interestingly, the ORR was 7.4 %, the disease control rate was 52 %, and the median OS was 16.4 months. Although this was a relatively small study, the results of this trial are highly comparable to the results of large-scale randomized studies in NSCLC patients that have been treated with docetaxel at doses of 75 mg/m^2 in a 3-week design [33–35]. Therefore, LDM docetaxel seems to be active as well. Whether this way of administration of docetaxel outmatches the conventional one with high doses of the drug every 2 or 3 weeks remains questionable. Thus, it is understandable why several researchers suggest that further investigation is required to elucidate the precise role of LDM docetaxel in the treatment of NSCLC patients.

In a phase I study that tested the combination of vinorelbine with cisplatin [23], 26 pretreated patients with NSCLC were enrolled and received per os vinorelbine three times per week at escalating doses ranging from 40 to 70 mg continuously plus intravenously cisplatin at escalating doses ranging from 70 to 85 mg/m^2 on day one of each cycle (cycles of 3 weeks). Maximum tolerated dose was determined at 60 mg for vinorelbine and at 85 mg/m^2 for cisplatin. The combination proved to be well tolerated, whereas hematological toxicity was the most common grade III–IV that occurred in seven patients (27 %). Among the 24 evaluable-for-response patients, five achieved partial response and ten patients stabilized their disease.

The results of LDM docetaxel that mentioned above formed the basis for the design of clinical studies using the same schedule of administration of docetaxel in patients with NSCLC. A study which recruited 21 patients with stage IV disease who had evidence of disease progression during or after first-line chemotherapy tested the simultaneous use of low doses of docetaxel at 25 mg/m^2 on days 1, 8, and 15 with 4-week cycles plus trofosfamide (a nitrogen mustard alkylating agent) 50 mg per day [25]. This combination proved well tolerated with manageable toxicity and quite effective. In particular, the overall response rate (ORR) was almost 20 %, whereas the median OS and PFS were 6.9 and 2.9 months, respectively. The estimated 1-year survival rate was 28.6 %.

Several chemotherapeutic agents have been also used in a metronomic administration. The efficacy and toxicity of chronic administration of oral etoposide were evaluated in 61 patients with inoperable NSCLC, in both first- and second-line settings. Etoposide was given orally, 100 mg daily for seven consecutive days and consequently 100 mg every other day for 14 additional days in a 28-day schedule. Toxicity was generally, acceptable. Myelotoxicity was the most common toxicity, particularly leukopenia, which was severe (grade 3 or 4) in nine patients (15 %). Seventeen patients (28 %) and 21 (34 %) achieved PR and SD, respectively. The median OS for all patients was 9 months, whereas the median OS for responders and nonresponders was 22 and 7 months, respectively [28]. The metronomic combination of cisplatin with vinorelbine has shown significant efficacy and favorable toxicity profile [23], but it is not clear what is the role of the combination of cisplatin with other regimens. Given that cisplatin is a key drug in the treatment of NSCLC and cisplatin-based doublets represent the backbone chemotherapy regimen in the frontline treatment of advanced NSCLC, the combination of cisplatin with other

chemotherapeutic agents administered in a metronomic schedule seems very attractive [23]. Indeed, the combination of weekly platinum 30 mg/m^2 on days 1, 8, and 14 in cycles of 4 weeks plus daily oral etoposide 50 mg/m^2 on 21 of 28 days was examined in a phase II trial. The mean time to progression and OS were 9 and 13 months, respectively, while the ORR was calculated at 45.2 % (two complete and 12 partial responses). The most common adverse events of that regimen were grade III leucopenia and anemia; three patients died due to pulmonary embolism. The message from this study was that the regimen is active even in patients with a very poor prognosis.

Conclusion

Metronomic therapy has shown very promising results in certain studies in NSCLC. Nevertheless, it seems unlikely that the administration of metronomic single-agent chemotherapy could lead to a long-lasting efficacy. There are indications that a combination with conventional therapy could be more active and less toxic. However, the most appropriate combination has not been determined yet. Further clinical research is needed in the future, to define the best chemotherapeutic combinations, the drug doses, and the duration of the treatment course. In addition, as demonstrated from the trials mentioned above, a better identification of the NSCLC patients who are more likely to benefit from such treatment is required. Since setting of antiangiogenic predictive markers remains an intractable problem in oncology today, other surrogate markers should be sought for the selection of these patients.

The whole theory for the development of metronomic therapy relied on the belief that vascular endothelial cells are stable with lack of genetic alterations and thus unable to develop resistance to the treatment. Unfortunately, nowadays, this rationale has been proved, at least in part, incorrect, since it is well known that tumor endothelial cells are completely different from the normal endothelial cells and commonly harboring genetic abnormalities [36, 37].

The advances that have been made during the last years in tumor, especially in NSCLC immunotherapy (e.g., BLP-25, anti-PD1 and anti-PDL1 agents), bring back to the surface the potential important role of metronomic chemotherapy as an immune stimulatory factor. Inhibition of the immune suppressive activity of T_{REGS} cells and subsequent restoration of normal immunity is displayed as a putative mechanism of its action. Of note, prospective trials, to this direction, are eagerly required in order to validate this very attractive hypothesis.

Whether other mechanisms, such as tumor dormancy, are involved in the mode of action of metronomic therapy has to be proved. However, Folkman and co-workers have shown that avascular tumors can be maintained in a dormant state [38]. Therefore, inhibiting tumor angiogenesis through the administration of metronomic chemotherapy may be able to induce and maintain dormancy. To demonstrate if this issue is true, prospective studies are needed using the most modern techniques in the searching of these dormant circulating tumor cells.

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Part III

Clinical Pharmacology of Metronomic Chemotherapy

Pharmacokinetics and Pharmacogenetics of Metronomic Chemotherapy

16

Guido Bocci and Giulio Francia

Abstract

Despite the numerous preclinical and clinical studies that have been conducted on metronomic chemotherapy in the past 10 years, few pharmacokinetic and pharmacogenetics data on this dosing regimen are available. Indeed, only the pharmacokinetics of metronomically administered drugs, such as irinotecan, UFT, and vinorelbine, have been described in patients, but no data are available on the most widely explored agents in such an approach like cyclophosphamide or capecitabine. Methodological issues and the neglected importance of the relationship between plasma concentrations of metronomically administered chemotherapeutic drugs (and their active metabolites) contributed to the absence of data on the commonly used 50 mg/day cyclophosphamide schedule. Moreover, few data are available on the pharmacogenetics of metronomic chemotherapy, and, although some objective responses have been obtained in various tumors, it remains largely unknown which genetic backgrounds could affect or predict the clinical response of patients. Trials integrating pharmacokinetic and pharmacogenetics research are necessary to better evaluate the clinical benefit of metronomic chemotherapy.

16.1 Introduction

The behavior and characteristics of chemotherapeutic drugs are quite diverse. The study of pharmacokinetics (the dose–concentration relationship) and pharmacodynamics (the concentration–response relationship) of chemotherapeutic drugs reveals

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this diverse behavior and sheds light on the different patterns of drug action. The knowledge base in pharmacokinetics and pharmacodynamics has grown considerably over the last years, and it has enabled seemingly counterintuitive concentration–response relationships to be understood (e.g., the antiangiogenic activity of low-dose metronomic chemotherapeutic drugs). The combination of the pharmacokinetic characteristics with the pharmacodynamic properties of a chemotherapeutic drug response may provide an almost complete knowledge of the dose–response relationship and, above all, can allow one to estimate the possible drug response at any dose, including at metronomic doses.

Chemotherapeutic drug action begins with the administration of the compound and concludes with the pharmacological response, which can be a beneficial and/or an adverse reaction. The dose, the frequency of administration, and the route of administration can permit the optimization of the onset, intensity, duration, and quality of therapeutic effects for a particular tumor type and the minimization of any harmful effects of the drug [1]. Thus, the design of optimum dosing regimens requires a deep understanding of the processes and of the steps that translate the administration of the drug into the pharmacological response. It also requires an understanding of how the administration–response relationship may be influenced by patient characteristics, as well as other conditions that may appear during the chemotherapy regimen. These include the age and gender of the patient, genetic factors (i.e., single-nucleotide polymorphisms) [2], concurrent medications, and changes in the tumor population being treated over time (i.e., onset of resistance) [3].

Using pharmacokinetics and pharmacogenetics, it should be possible to identify patients who will respond better to therapy and those at risk of rapidly developing drug resistance or of suffering from significant toxicity. In this regard, it needs to be pointed out that the dosing of metronomic chemotherapy remains largely empirical in the absence of validated clinical surrogate markers and pharmacokinetic drug monitoring for the treatment effects. Such pharmacokinetic data and pharmacodynamic markers are emerging in early-phase, pilot clinical studies (e.g., AUCs and circulating endothelial progenitor cells) [4–6], but their value in randomized phase III clinical studies remains questionable.

16.2 Pharmacokinetics of Metronomic Chemotherapy Regimens

Despite the growing amount of preclinical studies and clinical trials that have been conducted in the past 10 years [7, 8], few pharmacokinetic data of these schedules are currently available. Indeed, at the moment, only the pharmacokinetics of metronomic irinotecan, topotecan, vinorelbine, UFT, paclitaxel, and temozolomide have been described in patients [4, 9–14], but no data about the most widely explored agents in such an approach like cyclophosphamide or capecitabine have been provided. This lack of information may limit the clinical use and the efficacy of the metronomic regimens. Moreover, the variability in any of the pharmacokinetic

parameters (e.g., the peak concentration) may affect the impact of the drug. This can typically be observed in patients with organ dysfunction where the inability to either metabolize or excrete the drug will lead to unexpected drug effects [1]. A good pharmacokinetic assessment of the drugs administered metronomically is therefore the first and most important step in designing an individual treatment regimen that will maximize the antitumor drug benefit.

16.2.1 Metronomic Camptothecins

16.2.1.1 Irinotecan

Despite abundant information about the pharmacology of irinotecan [15], and of its active metabolite SN-38, on cancer cells using different therapeutic approaches, no data were available on the clinical effects of metronomic irinotecan administration until 2008. The pharmacokinetics of metronomic irinotecan (and its active metabolite SN-38) was performed, for the first time, in twenty patients with metastatic colorectal carcinoma, heavily pretreated both with irinotecan- and oxaliplatin-based chemotherapies at different dose levels [9]. This pilot study was defined and based on a previous pharmacokinetic experience with infusional schedule of irinotecan published by Falcone and colleagues [16]. The three different dose levels of metronomic irinotecan (infused continuously without breaks) were chosen starting from a reduction of 75 % of the maximum tolerable dose of irinotecan when infused continuously over 21 days every 28 days, as reported by Herben et al. [17]. The sample size ranged from a minimum of five to a maximum of eight patients *per* group which was sufficient to find a statistical difference between SN-38 (the active metabolite of irinotecan) pharmacokinetic parameters of each dose level [9]. The main pharmacokinetic parameters of irinotecan and its metabolites are reported in Table 16.1, whereas the mean plasma profiles of irinotecan, SN-38, and SN-38-glucuronide (the inactive form of SN-38) at the different infusion schedules are shown in Fig. 16.1a–c, respectively. Pharmacokinetic analysis demonstrated that the concentration at the steady state (C_{ss}) of SN-38 ranged from 1.00 ± 0.52 to 3.33 ± 0.96 ng/ml and was compatible with the antiangiogenic concentrations found in preclinical studies [18]. As expected, the C_{ss} of SN-38-glucuronide were higher than the ones of SN-38. Moreover, pharmacokinetic analysis showed an increased metabolism of irinotecan into the active metabolite SN-38 when higher doses were administered (Fig. 16.1b), a clear indication that such a process at these dose levels was not saturated. Thus, the mean AUC value of SN-38 was significantly lower at the irinotecan 1.4 mg/m^2/day dose than at the 2.8 and 4.2 mg/m^2/day doses (Table 16.1), and significant differences were found between the C_{max} values of SN-38 and SN-38glu at different irinotecan doses [9].

16.2.1.2 Topotecan

Topotecan has excellent antiangiogenic properties when administered on a metronomic schedule in preclinical models [19–22]. Various dosing schedules of oral topotecan have been evaluated in phase I studies, establishing the maximum

Table 16.1 Pharmacokinetic parameters of irinotecan, SN-38, and SN-38-glucuronide at the doses of irinotecan 1.4, 2.8, and 4.2 mg/m^2/day in 20 colorectal cancer patients

	Mean ± SD		
	1.4 mg/m^2/day (n = 7)	2.8 mg/m^2/day (n = 5)	4.2 mg/m^2/day (n = 8)
Irinotecan			
AUC (day·ng/ml)	8,714.7 ± 1,564.3	13,877.7 ± 3,035.2	23,051.6 ± 5,002.3
CL (ml/day/m^2)	154.32 ± 28.4	170.31 ± 44.2	146.11 ± 25.3
$t_{1/2}\beta$ (h)	15.9 ± 5.1	20.2 ± 6.2	14.6 ± 3.2
C_{max} (ng/ml)	277.6 ± 125.3	382.9 ± 261.8	484.1 ± 243.1
C_{ss} (ng/ml)	143.1 ± 56.8	231.6 ± 101.4	390.0 ± 171.0[a,b]
T_{max} (day)	35	35	28
SN-38			
AUC (day·ng/ml)	59.43 ± 7.47	136.21 ± 10.61[c]	200.48 ± 12.26[a,b]
$t_{1/2}\beta$ (h)	18.9 ± 4.3	22.8 ± 6.7	19.9 ± 7.2
C_{max} (ng/ml)	1.62 ± 0.45	2.61 ± 1.07	4.03 ± 2.19[a]
C_{ss} (ng/ml)	1.00 ± 0.52	2.29 ± 0.87[c]	3.33 ± 0.96[a,b]
T_{max} (day)	42	35	35
SN-38-glucuronide			
AUC (day·ng/ml)	100.94 ± 8.82	268.86 ± 14.52[c]	430.10 ± 24.34[a,b]
$t_{1/2}\beta$ (h)	22.31 ± 5.1	17.4 ± 5.6	21.33 ± 6.8
C_{max} (ng/ml)	2.24 ± 0.58	5.59 ± 1.91[c]	8.45 ± 2.54[a, b]
C_{ss} (ng/ml)	1.63 ± 0.53	4.42 ± 1.98[c]	7.20 ± 1.59[a,b]
T_{max} (day)	49	42	42

AUC area under the time/concentration curve, $t_{1/2}\beta$ terminal half-life, C_{max} maximal plasma concentration, T_{max} time to peak, C_{ss} plasma concentration at the steady state

[a] $P < 0.05$ 4.2 vs. 1.4

[b] $P < 0.05$ 4.2 vs. 2.8

[c] $P < 0.05$ 2.8 vs. 1.4

tolerated dose to a 1.0 mg fixed daily dose for a metronomic regimen [23]. Indeed, Tillmanns and colleagues enrolled 16 heavily pretreated patients with various solid tumors in a phase I dose-ranging study consisted of 30-day treatment cycles of daily oral topotecan at dose levels of 0.25, 0.50, 0.75, 1.00, and 1.25 mg [23]. The dose-limiting toxicity was reached at 1.25 mg (i.e., two patients had grade 3 gastrointestinal toxicities), and the maximum tolerated dose was defined at 1.0 mg daily [23]. Interestingly, as previously noted for irinotecan, the topotecan C_{max} increased linearly with the dose and the median T_{max} was 2 h [23]. On the basis of these preliminary results, the same group implemented a combination of metronomic oral topotecan (0.25 mg daily) and oral pazopanib (400, 600, or 800 mg daily) in a phase I, dose-escalation study in female patients with gynecological tumors [12]. A preclinical pharmacokinetic study of metronomic topotecan plus pazopanib suggested the absence of drug–drug interactions [20]. The published clinical data supported these preclinical findings, although a large inter- and intrapatient variability was observed [12]. The median of topotecan *Cl*/*F*, *Vc*/*F*, and *k*a were 26.7 l/h, 144 l, and 1.04 h^{-1}, respectively. A mean topotecan C_{max} around 1 ng/ml was found at the 0.25 mg dose schedule, and the onset of the absorptive phase was delayed for several patients [12]. The authors indicated that a one-compartment model with

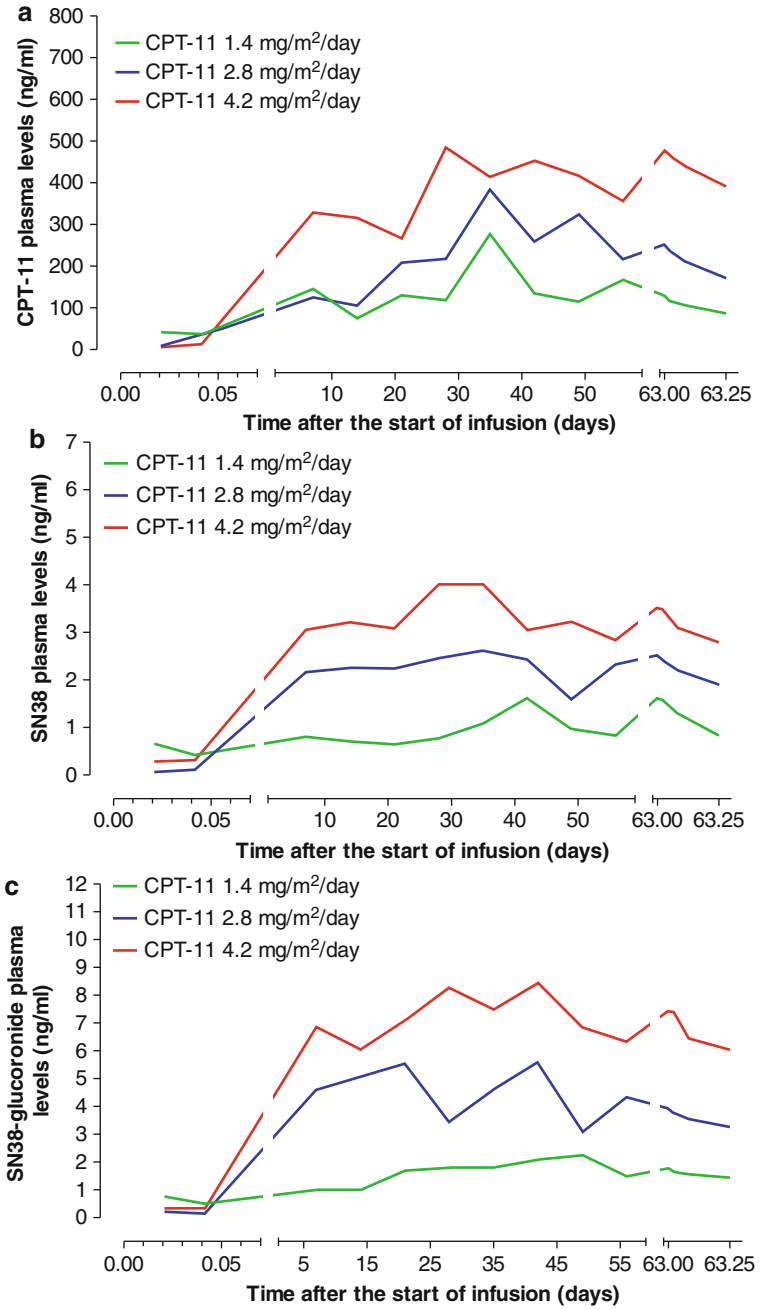

Fig. 16.1 Plasma levels of irinotecan (CPT-11) (**a**), SN-38 (**b**), and SN-38 glucuronide (**c**) in 20 mCRC patients receiving an i.v. continuous infusion of CPT-11 at three different dose levels. The continuous line represents the mean plasma concentrations

first-order absorption/lag-time and linear elimination from the central compartment adequately described the topotecan plasma concentrations vs. time profiles. Interestingly, a recent study suggested that patients receiving higher topotecan doses may develop pharmacokinetic interactions with this combination [24]. Thus, the advantage of the metronomic schedule is that it may avoid unfavorable drug interactions that are likely dose dependent.

16.2.2 Metronomic Microtubule-Binding Agents

16.2.2.1 Vinorelbine

Microtubule-binding agents have been suggested to be the most promising cytotoxic drugs for metronomic administration because of their ability to suppress microtubule dynamics and interfere with endothelial cell functionality at very low concentrations [25–27].

Oral vinorelbine, a semisynthetic vinca alkaloid with antimicrotubule activity, has been administered metronomically in clinical studies on lung and breast cancers [10, 28–30]. The availability of this oral formulation (soft caps) is clearly advantageous for chronic, metronomic administration. The pharmacokinetics of oral vinorelbine at standard doses has been described as linear with a moderate interpatient variability, showing a bioavailability of 40 %, which is not influenced by food or age. Oral vinorelbine is rapidly absorbed (1.5–3 h) with an elimination half-life of approximately 40 h, and it shows a low level of binding to plasma proteins (13 %), whereas it is highly bound to platelets (78 %). Oral vinorelbine is metabolized mainly in the liver by the CYP3A4 isoform and eliminated mainly in an unconjugated form via the bile [31].

The pharmacokinetics of metronomic oral vinorelbine were described by Briasoulis and colleagues in 2009 [11]. In this open-label, ascending-dose (from 20 to 70 mg given thrice a week) trial, 62 patients were enrolled, but only 37 were tested for vinorelbine blood concentrations and included into the pharmacokinetic evaluation. Samples were collected after 14 days from the beginning of the treatment and up to 5 months after the beginning of metronomic regimen. Also low-dose vinorelbine showed linear pharmacokinetics with a constant concentration/dose ratio and a proportional increase of concentrations for escalating administered doses. Moreover, the blood concentration steady state for both vinorelbine and its active metabolite 4-O-deacetylvinorelbine was achieved after 2 weeks of treatment, and it was stable for months, ranging from 0.5 to 1.5 ng/ml [11]. Interestingly, the achieved steady-state concentrations were consistent with the previous in vitro findings evaluating the optimal inhibition of endothelial cell proliferation [32], supporting the hypothesis that the chosen schedule of oral vinorelbine was able to attain protracted, very low, but cytotoxic concentrations for endothelial cells [11]. In 2013, the results of a multi-institutional randomized open-label phase IB trial were published by the same group [10]. Seventy-three patients were randomly assigned to 30, 40, or 50 mg vinorelbine, taken orally three times a week, and the pharmacokinetics of the drug was performed. Trough levels of vinorelbine were measured in

blood samples from 44 patients over a time that ranged from 2 to 36 weeks. Steady-state concentrations were similar to those previously obtained [11], with no evidence of accumulation over time. Indeed, the measured mean concentration values were 1.8 ± 1.10 ng/ml (for the 30 mg dose), 2.2 ± 1.87 ng/ml (40 mg), and 2.6 ± 0.69 ng/ml for the 50 mg dose [10]. Thus, both vinorelbine and its active metabolite achieved steady-state concentrations at the low nanomolar range, which were found in vitro to preferentially inhibit the proliferation of endothelial cell and induce the expression of endogenous antiangiogenic molecules.

16.2.2.2 Paclitaxel

At standard doses, paclitaxel binds to the beta-subunit of polymerized tubulin and inhibits the dissociation rate of the tubulin subunits from the tubule. Besides these known pharmacodynamic properties, paclitaxel also exhibits antiangiogenic activity [27]. In the last decade, numerous efforts have focused on improving the pharmacokinetic behavior of paclitaxel, synthesizing a variety of nanoparticle carrier systems such as liposomes, pegylated liposomes, proteins, and polymeric nanoparticles [33].

The antitumor and antiangiogenic effects of metronomic cyclic NGR (Asn–Gly–Arg)-modified liposomes containing paclitaxel (NGR-SSL-PTX) have been recently demonstrated in a preclinical model of HT1080 (human fibrosarcoma cells) tumor-bearing SD rats in vivo [34]. Thus, Luo and colleagues performed a pharmacokinetic study of metronomic NGR-SSL-PTX in a subgroup of rats, showing that paclitaxel in NGR-SSL-PTX was more slowly eliminated from the circulation. The value of the mean residence time and the elimination half-life in the NGR-SSL-PTX treatment groups significantly increased if compared with those in the standard paclitaxel treatment group. Furthermore, the bioavailability and the AUC values were significantly increased in the NGR-SSL-PTX treatment groups, whereas the clearance of paclitaxel in the NGR-SSL-PTX treatment groups was significantly lower [34].

Recently, an oral solid dispersion formulation (ModraPac001) of paclitaxel for use in low-dose metronomic chemotherapy was clinically tested in a proof-of-concept clinical study [13]. Over a period of 2 weeks, four patients received once a week 30 mg paclitaxel p.o. and 100 mg ritonavir p.o. Paclitaxel was formulated as a solid dispersion formulation (ModraPac001, 10 mg capsule) or as a premix solution. In this study, the paclitaxel mean peak plasma concentration (C_{max}) after weekly administration of 30 mg ModraPac001 was 41.8 ng/ml; but after 24 and 48 h, the plasma concentrations were 1.67 ± 098 ng/ml and 0.80 ± 0.72 ng/ml, respectively [13]. Interestingly, these concentrations resulted well within the anti-endothelial range of paclitaxel showed by the studies of Bocci et al. [35] and Wang et al. [36] and below the myelosuppression threshold of 43 ng/ml established by Gianni and colleagues [37].

16.2.3 Metronomic UFT

UFT, a combination of tegafur, a prodrug of 5-fluorouracil (5-FU), and uracil, has demonstrated clinical activity in many tumors and, in particular, in the treatment of gastrointestinal cancers [38, 39]. It has been successfully tested using low-dose

Table 16.2 Pharmacokinetic parameters of tegafur, 5-FU, 5-FUH_2, GHB, and uracil in 27 patients administered with metronomic UFT, cyclophosphamide, and celecoxib

Parameter (u nits)	Day 1	Day 28	Day 56
Pharmacokinetic parameters for FT			
AUC (h·μg/ml)	6.286[a,b] ± 5.976	15.25 ± 9.953	12.50 ± 11.04
T_{max} (h)	1.160 ± 1.405	1.479 ± 1.536	1.188 ± 1.232
C_{max} (μg/ml)	1.976[a,b] ± 1.916	4.342 ± 2.516	3.458 ± 2.965
Pharmacokinetic parameters for 5-FU			
AUC (h·μg/ml)	1.735 ± 1.712	2.221 ± 2.444	2.192 ± 2.249
T_{max} (h)	1.240 ± 1.473	1.196 ± 1.126	1.306 ± 1.296
C_{max} (μg/ml)	0.734 ± 0.732	0.851 ± 0.817	0.918 ± 1.008
Pharmacokinetic parameters for 5-FUH2			
AUC (h·μg/ml)	2.462 ± 2.368	2.884 ± 2.041	2.998 ± 1.682
T_{max} (h)	1.180 ± 0.912	1.217 ± 0.877	1.342 ± 0.867
C_{max} (μg/ml)	1.151 ± 1.121	1.179 ± 1.506	1.625 ± 1.127
Pharmacokinetic parameters for uracil			
AUC (h·μg/ml)	5.437 ± 5.726	5.658 ± 5.192	5.192 ± 5.540
T_{max} (h)	0.920 ± 0.932	1.717 ± 1.744	1.500 ± 1.496
C_{max} (μg/ml)	2.710 ± 3.833	2.123 ± 1.835	2.182 ± 2.181
Pharmacokinetic parameters for GHB			
AUC (h·ng/ml)	500.9[a,b] ± 54.75	361.1 ± 48.05	395.1 ± 60.48
T_{max} (h)	1.880 ± 1.502	2.00 ± 1.559	1.925 ± 1.558
C_{max} (ng/ml)	161.7[a,b] ± 94.55	127.7 ± 72.25	128.2 ± 75.13

[a]day 1 vs. day 28
[b]day 1 vs. day 56
[c]day 28 vs. day 56

protocols in a randomized phase III adjuvant therapy trial of non-small cell lung cancer [40] where the drug was taken orally every day for at least 2 years.

The pharmacokinetics of metronomic UFT have been recently described by Allegrini and colleagues in a subset of metastatic, fluoropyrimidine-resistant patients with advanced refractory gastrointestinal cancers enrolled in a phase II clinical trial [4]. Furthermore, this study described a statistical relationship between pharmacokinetic parameters and the clinical efficacy of the metronomic chemotherapy. The therapeutic schedule was established using, on day one, a single administration of cyclophosphamide (CTX) 500 mg/m^2 as i.v. bolus and, from day two, administration of 50 mg cyclophosphamide p.o. once daily plus 100 mg UFT p.o. and 200 mg celecoxib (CXB) p.o. twice a day. From day two, the treatment was continued without interruption. The pharmacokinetic analyses of tegafur (FT), 5-fluorouracil (5-FU), 5-dihydro-5,6 fluorouracil (5-FUH_2), uracil, and GHB were performed in 27 patients of 38 enrolled at the days 1, 28, and 56 after the start of therapy (Fig. 16.3). A statistically significant difference in the values of area under curve (AUC) and maximum plasma concentration (C_{max}) on day 1 compared with those on day 28 and day 56 of tegafur and 5-FU was found (Table 16.2 and Fig. 16.2a, b). Moreover, after the first intake of 100 mg UFT tablet, the analysis revealed a significant difference between the pharmacokinetic parameters of patients in progressive disease (PD) and in stable disease (SD) in 5-FU AUC and C_{max} values on

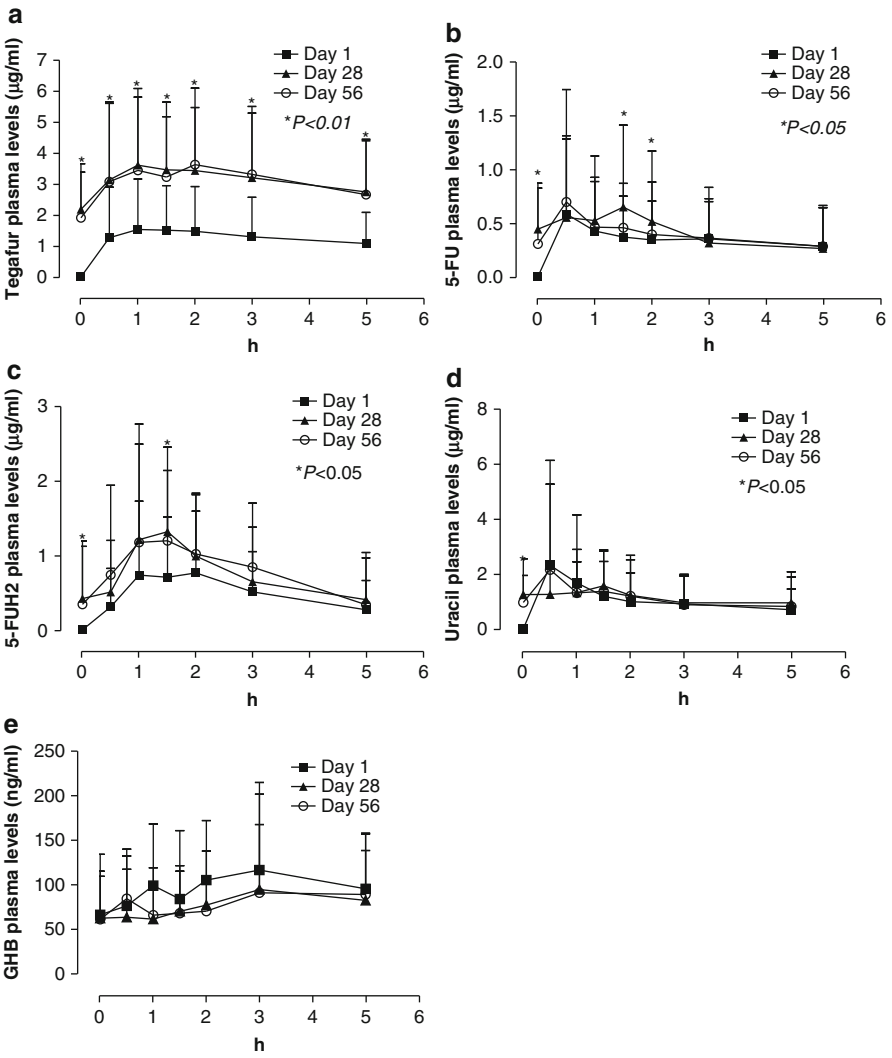

Fig. 16.2 Plasma levels of tegafur (**a**), 5-FU (**b**), 5-FUH_2 (**c**), uracil (**d**), and GHB (**e**), in 27 patients at days 1, 28, and 56, receiving the metronomic CTX, UFT, and CXB schedule. *Points* mean, *bars* standard deviation. *$P < 0.01$ and < 0.05 vs. day one values

day one (Table 16.3 and Fig. 16.3). Even more interesting, patients with the 5-FU AUC and C_{max} pharmacokinetic parameters at day one greater than the cut-off values of 1.313 h × µg/ml and 0.501 µg/ml, respectively, showed a significant prolonged progression-free survival and a significant increase in overall survival [4].

Despite the limitation of this analysis due to the small number of patients, these results identified a pharmacokinetic cut-off value in a clinically relevant population and may reveal how UFT pharmacokinetic parameters may be used from the very

Table 16.3 Pharmacokinetic parameters of tegafur, 5-FU, 5-FUH_2, GHB, and uracil in 13 patients with stable disease (SD) and 14 patients with progressive disease (PD) administered with metronomic UFT, cyclophosphamide, and celecoxib

Parameter (units)	Day 1	Day 28	Day 56
Pharmacokinetic parameters for FT (PD)			
AUC (h·μg/ml)	5.380[b] ± 6.701	17.01 ± 11.66	10.24 ± 12.72
T_{max} (h)	1.464 ± 1.770	1.833 ± 1.614	1.375 ± 1.597
C_{max} (μg/ml)	1.602[b] ± 2.122	4.900 ± 2.918	2.668 ± 3.287
Pharmacokinetic parameters for FT (SD)			
AUC (h·μg/ml)	6.295[b,c] ± 5.330	13.49 ± 8.027	14.75 ± 9.055
T_{max} (h)	0.654[c] ± 0.625	1.125 ± 1.432	1.000 ± 0.738
C_{max} (μg/ml)	2.076 ± 1.713	3.784 ± 2.009	4.249 ± 2.494
Pharmacokinetic parameters for 5-FU (PD)			
AUC (h·μg/ml)	0.997[a] ± 1.271	1.307[a] ±1.109	1.916 ± 1.702
T_{max} (h)	1.308[a] ± 1.588	1.333 ± 1.420	0.714 ± 0.636
C_{max} (μg/ml)	0.453 ± 0.573	0.542 ± 0.737	0.887 ± 1.314
Pharmacokinetic parameters for 5-FU (SD)			
AUC (h·μg/ml)	2.765 ± 1.709	3.514[d] ± 2.875	2.369 ± 2.602
T_{max} (h)	1.273 ± 1.421	1.045 ± 0.723	1.682 ± 1.488
C_{max} (μg/ml)	1.134 ± 0.749	1.188 ± 0.795	0.938 ± 0.832
Pharmacokinetic parameters for 5-FUH2(PD)			
AUC (h·μg/ml)	2.053 ± 2.361	3.170 ± 2.422	2.971 ± 1.974
T_{max} (h)	1.143[b,c] ± 1.117	1.333 ± 0.835	1.563 ± 1.116
C_{max} (μg/ml)	0.903 ± 1.027	2.111 ± 1.898	1.887 ± 1.496
Pharmacokinetic parameters for 5-FUH2 (SD)			
AUC (h·μg/ml)	2.983 ± 2.382	2.573 ± 1.582	3.018 ± 1.537
T_{max} (h)	1.227 ± 0.607	1.091 ± 0.944	1.182 ± 0.643
C_{max} (μg/ml)	1.466 ± 1.203	1.291 ± 0.802	1.435 ± 0.790
Pharmacokinetic parameters for uracil (PD)			
AUC (h·μg/ml)	4.217 ± 4.810	4.343 ± 5.037	4.855 ± 6.064
T_{max} (h)	0.893 ± 1.022	1.625 ± 1.760	1.750 ± 1.648
C_{max} (μg/ml)	1.928 ± 2.368	1.597 ± 1.618	1.479 ± 1.586
Pharmacokinetic parameters for uracil (SD)			
AUC (h·μg/ml)	6.989 ± 6.625	7.093 ± 5.201	5.417 ± 5.429
T_{max} (h)	0.954 ± 0.850	1.818 ± 1.807	1.333 ± 1.435
C_{max} (μg/ml)	3.705 ± 5.101	2.697 ± 1.957	2.651 ± 2.453
Pharmacokinetic parameters for GHB (PD)			
AUC (h·ng/ml)	491.5 ± 302.2	303.1 ± 256.5	422.5 ± 235.3
T_{max} (h)	2.143 ± 1.537	1.625 ± 1.479	2.25 ± 0.9258
C_{max} (ng/ml)	165.9 ± 114	107.7 ± 75.35	144.8 ± 61.98
Pharmacokinetic parameters for GHB (SD)			
AUC (h·ng/ml)	512.9 ± 74.34	424.4 ± 20.21	376.9 ± 86.7
T_{max} (h)	0.5667 ± 2.524	1.328 ± 3.49	0.230 ± 0.753
C_{max} (ng/ml)	111.4 ± 201.5	105.7 ± 193.2	64.03 ± 170.1

[a]PD vs. SD
[b]day 1 vs. day 28
[c]day 1 vs.day 56
[d]day 28 vs. day 56

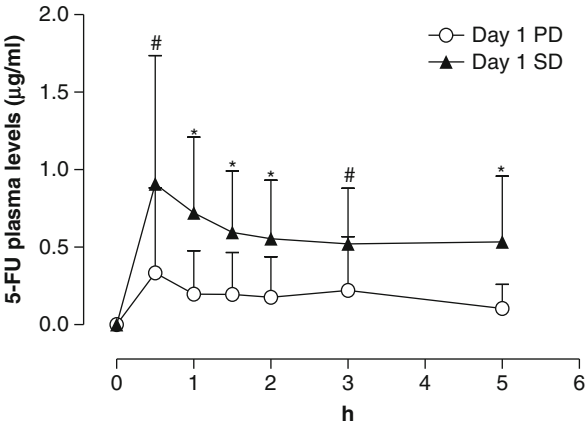

Fig. 16.3 Plasma levels of 5-FU in 13 stable disease (*SD*) patients and 14 progressive disease (*PD*) patients at day 1, 28, and 56, receiving the metronomic CTX, UFT, and CXB schedule. *Points* mean, *bars* standard deviation, $^{*}P<0.05$ PD vs. SD, $^{\#}P<0.01$ PD vs. SD

first administration of the drug to predict the efficacy and the survival of colorectal patients undertaking the metronomic schedule. Interestingly, as in the case of other chemotherapeutic drugs, the observed 5-FU concentrations are far lower than those that can be achieved with conventional 5-FU chemotherapeutic schedules.

16.2.4 Metronomic Alkylating Agents

Although alkylating drugs such as cyclophosphamide (CTX) and temozolomide (TMZ) are among the most commonly used compounds for metronomic regimens administered in the clinic to treat various tumor types such as breast, prostate, and brain cancers [41, 42], few clinical pharmacokinetic data are currently available. Reasons for this include methodological issues such as long-term sampling or low-sensitivity detection methods. They also include the neglect of the importance of the relationship between plasma concentrations of metronomic drugs (and their active metabolites) and clinical activity. This has consequently led to the absence of such data for the commonly used 50 mg/day cyclophosphamide schedule.

16.2.4.1 Cyclophosphamide

The preclinical pharmacokinetics of metronomic cyclophosphamide were investigated in xenotransplanted mice and in tumor-free animals of the same strain [43]. The concentrations of one active metabolite of cyclophosphamide (i.e., 4-OH-cyclophosphamide) were measured in the blood of three different mouse strains that were continuously given 20 mg/kg/day of cyclophosphamide through the drinking water for up to 8 weeks [44]. The authors found that the steady-state 4-OH-CTX concentrations were reached after 1 week and that the active metabolite levels measured after 8 weeks of metronomic administration were similar to those after 1 week of treatment, suggesting the absence of accumulation phenomena. The variability in AUC and C_{max} values among the mouse strains was ascribed to the interstrain heterogeneity of CTX biotransformation. Of note, the presence

of PC-3 xenografts resulted in decreased 4-OH-CTX concentrations in nude mice compared with tumor-free animals of the same strain [43].

Currently, no clinical data are available on metronomic CTX pharmacokinetics in adult patients. Adenis and colleagues recently performed a 3 + 3 dose-escalating phase I trial with a fixed dose of metronomic cyclophosphamide (50 mg two times daily) plus imatinib (400 mg per day; 300 and 400 mg two times daily), studying the imatinib pharmacokinetic parameters. The authors concluded that no dose-limiting toxicity and no drug–drug pharmacokinetic interaction were observed [45].

16.2.4.2 Temozolomide

TMZ is rapidly and well absorbed after oral administration, and it undergoes spontaneous hydrolysis at physiological pH to form its active metabolite, 3-methyl-(triazen-1-yl) imidazole-4-carboxamide (MTIC), which further degrades to 5(4)-aminoimidazole-4(5)-carboxamide and a highly reactive methyl-diazonium cation. Zhou and colleagues made a comparative pharmacokinetic study in nude rats using conventional and metronomic doses of TMZ to provide a foundation for the design of optimal metronomic TMZ treatments [46]. The pharmacokinetics of TMZ appeared linear and both dose and time independent, as there were no differences between the systemic clearance and the volume of distribution in the conventional and metronomic dose groups on the first day and the last treatment day. The ratio of the mean AUC values on day one in the conventional dose group to those in the metronomic dose group was 5.6, which was identical to the dose ratio. In addition, the $t_{1/2}$ of TMZ remained essentially the same, independently from the dose and time of sampling. Interestingly, the authors discovered that there were no sustainable changes in tumor accumulation of the drug between the conventional and metronomic dose regimens [46].

Baruchel and colleagues demonstrated in 2006 the feasibility and safety of administering metronomic TMZ in pediatric cancer patients, and they also determined a TMZ pharmacokinetic profile in these children [14]. The pharmacokinetic study was conducted in 19 patients on day one of the first cycle at various time points after the TMZ dose. The peak concentration and the area under the curve increased with increasing doses, and TMZ at metronomic doses showed a linear pharmacokinetics, although with an important interpatient variability due to a limited sample size in each cohort and various dose levels. The C_{max} ranged from a value of 2.42 ± 0.61 mg/l after the administration of 50 mg/m^2 TMZ dose to 3.51 ± 1.26 mg/l after 100 mg/m^2 TMZ dose where the AUC varied from 10.66 ± 7.70 to 13.66 ± 4.64 mg/l·h, respectively [14]. No correlation was observed between pharmacokinetic data (AUC and peak concentration) and toxicity of or response to TMZ.

16.2.5 Future Perspectives on Pharmacokinetics of Metronomic Chemotherapy

The lack of a well-known pharmacokinetic profile represents the “dark side” of metronomic chemotherapy regimens and makes it impossible to determine, among other things, (i) an optimal biological dose, (ii) the correct dose reduction vs. the

conventional schedules, and (iii) any possible pharmacokinetic interactions with other drugs, such as tyrosine kinase inhibitors, or therapeutic antibodies that target angiogenic proteins.

Moreover, if significant research effort is not devoted to this specific area of pharmacology research, it will be impossible to (i) determine the main mechanisms of action involved in the success of metronomic chemotherapy at a specific range of drug concentrations in plasma or (ii) identify valid pharmacodynamic markers of the therapy in oncology patients for such drug concentrations. Indeed, although some objective responses have been obtained in various tumors, it remains mainly unknown which plasma concentrations of the drugs were attained in the responding subjects. This makes it difficult to objectively evaluate the value of the metronomic administration of chemotherapeutic drugs. Thus, randomized clinical trials that integrate pharmacokinetic analysis are absolutely essential to better evaluate the clinical benefit of metronomic chemotherapy for the palliative treatment of cancer.

16.3 Pharmacogenetics of Metronomic Chemotherapy

Optimum drug administration is important not only for ensuring good patient outcomes in clinical practice but also in the design of clinical trials during drug development. The costs of the clinical development of a new drug or a novel therapeutic regimen are enormous, and therefore, it is critical that all drug candidates selected for human trials should be evaluated in the most efficient, cost-effective manner. With drugs used in the field of metronomic chemotherapy having unknown therapeutic indices, it becomes imperative that we understand the mechanisms behind the observed variability in drug response when treating a cancer patient.

Pharmacogenetics, an important component of individualized therapy in cancer patients, focuses on describing the extent to which an individual's genetic background is responsible for the observed differences in drug efficacy and toxicity profiles [2]. This information is then used to make predictions about the toxicity and efficacy of chemotherapeutic drugs in patients. Inherited variability of drug targets, drug-metabolizing enzymes, and drug transporters may all have a major impact on overall drug response, disposition, and associated adverse drug reactions by altering the pharmacokinetic and pharmacodynamic properties of chemotherapeutic drugs [47]. Single-nucleotide polymorphisms (SNPs) are the simplest and most commonly studied DNA polymorphism that occurs in the human genome, and they account for more than 90 % of the genetic variation observed between individuals.

Although metronomic chemotherapy has been used for over a decade in patients, it has not yet been investigated from a pharmacogenetics perspective, with the exception of two pilot studies [48, 49]. Indeed, new pharmacogenetics approaches to predict the clinical effects of metronomic chemotherapy regimens and the survival of patients are urgently needed in order to improve the personalization of this therapy for cancer patients. The role of the tumor microenvironment in the response to antitumor therapies is being increasingly emphasized [50]. Indeed, the individual genetic background of patients could have an important role in the responses to

chemotherapeutic drugs or to antiangiogenic agents, such as metronomic chemotherapy, by modulating the secretion of pro-angiogenic factors (e.g., IL-8 or VEGF) or of endogenous angiogenesis inhibitors (e.g., TSP-1). Schultheis and colleagues performed a very interesting research on 70 recurrent/metastatic ovarian cancer patients, who were treated with metronomic CTX and bevacizumab [49]. Patients harboring the IL-8 + 251 AA or AT genotypes had a significantly lower response rate than those with the TT genotype, whereas patients with the VEGF-A +936CT genotype showed a trend (although not statistically significant) for longer median progression-free survival, compared with those with the TT genotype [49]. Thus, these results may suggest that the IL-8 251A/T polymorphism could be a molecular predictor of response for the combination therapy of metronomic CTX and bevacizumab.

A recent study focused on the VEGF-A gene and its genetic variants in order to evaluate their influence on the response and survival to metronomic CTX therapy in 43 patients with metastatic prostate cancer [48]. Orlandi and colleagues tested the hypothesis that VEGF-A functional polymorphisms could modulate the response of some prostate cancers to metronomic treatment. Therefore, the study of VEGF-A SNPs should help identify those patients that are susceptible to, or resistant to, metronomic therapy. In that study, in nonresponder patients, the –634CC VEGF-A genotype frequency was 22.73 %, whereas no patient with CC genotype was observed in the responder's group ($P = 0.0485$). Moreover, the -2578CC VEGF-A genotype resulted more frequent (18.60 % vs. 2.33 %) in nonresponders ($P = 0.0212$). However, the most relevant finding of that pharmacogenetics pilot study was the identification of a VEGF-A genotype that was significantly associated with progression-free survival. Indeed, patients harboring the -634CC VEGF-A genotype had a median PFS of 2.2 months (95 % CI 0.45–3.95 months), whereas patients with genotype –634CG/GG VEGF-A had a median PFS of 6.25 months (95 % CI 3.28–8.62 months; $P = 0.0042$) [48]. Thus, a genetically determined modulation of VEGF-A in the tumor microenvironment could have a decisive role in the response of a tumor to metronomic therapy.

The validation of specific polymorphisms that will predict response to metronomic regimens is a complex process, which will need to involve both pilot and randomized clinical studies. At the present time, metronomic chemotherapy is mainly explored as a palliative treatment strategy after numerous lines of standard chemotherapy in phase II clinical trials and few phase III studies have been planned [41]. In that respect, the collaborative efforts of investigators who actively worked in this field will be particularly important in providing a wider series of patients to validate promising but preliminary results. For example, future phase III metronomic clinical trials should include analysis of a full coverage of genes and genetic variants of the IL-8 and VEGF-A pathways, based on the available preliminary data [48, 49]. Moreover, future studies should also include the analysis of SNPs of genes involved in the metabolism of chemotherapeutic drugs, such as CYP2B6, 3A4, and 2C9 involved the biotransformation of CTX into a 4-hydroxycyclophosphamide, the so-called activation step [51]. Indeed, SNPs that could enhance or decrease the enzymatic activity of the above-described CYPs may vary the tumor response to

metronomic chemotherapy and could therefore have an impact on the survival of patients treated with such regimens.

Pharmacogenetics analyses should be conducted as an integral part of large randomized phase II/III trials of metronomic chemotherapy or as independent studies that focus on the validation of specific genetic determinants. However, the introduction of pharmacogenetics of metronomic chemotherapy into clinical practice will be very difficult, even if candidate genes (e.g., IL-8 or VEGF-A) will be characterized. Indeed, studies aimed at documenting clinically the predictive efficacy of such SNPs, and a comparison of pharmacogenetically guided vs. standard patient care will also be necessary.

Conclusions

The field of metronomic chemotherapy adds another level of complexity to issues such as the characterization of clinically relevant pharmacokinetic parameters or the germ-line and somatic mutations that can affect drug efficacy. However, as we begin to unravel and accurately identify (i) the mechanism of action of metronomic anticancer drugs at the real plasma concentrations obtained from low-dose regimens and (ii) the polymorphisms in candidate genes likely to influence drug efficacy, we may start to consider the personalization of metronomic chemotherapy regimens for cancer patients.

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Metronomics: Potential Social Impact and New Business Models to Improve Availability of Cancer Treatments

17

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Abstract

Over the last 15 years, metronomic chemotherapy (MC) alone or combined with drug repositioning (DR) has gradually gained interest from cancer researchers and clinicians. Metronomics, the combination of MC and DR, can provide inexpensive, easy to administer and non-toxic treatments for cancer patients, while introducing innovative mechanisms of anti-tumour action.

In this article, we explore how the use of metronomics can deliver important social gain by allowing to treat patients for whom therapeutic options would be otherwise limited or absent. Thus, patients living in low- and middle-income

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countries (LMICs) can benefit from metronomics to the same extent as patients living in high-income countries (HICs). In both settings, frail patients can also take advantage of the low toxicity associated with metronomics. Here, we focus on new business models that could help in bridging the gap between LMICs and HICs with regard to anticancer treatments through the use of metronomics. In particular, we analyse the principles explaining how business model innovation can make cancer therapies available and affordable to patients with limited resources. Overall, we argue that new business models are essential to the optimal development of metronomics and the combination of business model innovation with metronomics can help fight cancer in LMICs.

17.1 Global Oncology

In 2008, roughly 70 % of cancer deaths occurred in low- and middle-income countries (LMICs) where survival rates are much lower [1]. Indeed, even though major breakthroughs have been made in basic research and cancer care in the last decades, the progress observed in high-income countries (HICs) – leading for instance to an increase of 36 % in 5-year survival rates between the periods of 1975–1977 and 1999–2006 in the USA for all tumour types – has not been extended to patients living in LMICs [2]. For many of these patients, most medical advances unfortunately remain hopes for the years to come. The discrepancy is even more dramatic for childhood cancer patients. Indeed, with 80 % of all children living in LMICs and based on estimated incidence and survival rates (approximately 200,000 new cases per year and 25 % survival in LMICs versus 50,000 new cases and 75 % survival in HICs), cancer is thought to kill approximately ten times more children in LMICs than in HICs. In developing countries, many patients do not even have access to cancer treatments and are ultimately sent home to die, and an even larger number of patients do not have access to treatment facilities at all [3]. New constraint-adapted therapeutic strategies are therefore urgently needed. Metronomic chemotherapy (MC) represents a genuine alternative for these patients [4]. Indeed, this low-cost, well-tolerated and easy to access strategy makes it a very attractive therapeutic option in resource-limited countries. Moreover, combined with drug repositioning, additional anticancer effects can be achieved with the metronomic approach [3].

Recently, global oncology [5] has emerged and been put on the forefront by several international agencies. For instance, in 2011, the United Nations held a high-level summit on the prevention and control of non-communicable diseases (NCD) and adopted resolution 66/2 [6, 7]. This resolution aimed both at increasing awareness and at leading/initiating efforts to fight NCDs globally including cancer, which causes more than 7.1 million deaths annually.

Besides its "human" cost, cancer also represents a major economic burden, with a total economic impact of US$895 billion in 2008. Furthermore, this amount is only based on the economic loss from lost years and productivity as estimated by a study supported by the American Cancer Society [1] and thus does not include direct costs of treatment. As an example, the economic weight of cancer was 20 %

higher than that of heart diseases, which represent the second-most common cause of NCDs [8].

Lower survival rates for patients living in LMICs result from several interconnected issues. First, local dedicated infrastructures are inferior, sometimes absent due to socio-economic restrictions, leading to a lethal combination of late stage at diagnoses and limited access to timely and effective treatment [9]. Second, cancer care is expensive and nowadays relies on complex technologies. Poor economies cannot currently access curative therapies, state-of-the-art surgery or expensive cancer drugs that constitute the backbones of cancer care in developed nations. Only 5 % of global cancer resources are currently spent in developing countries, which account for about 80 % of disability-adjusted life years lost in the world to this disease [1, 3]. The combination of both limited resources and late presentations results in high mortality rates. This in turn frequently leads to the misconception that nothing or little can be done for cancer patients living in developing countries [3]. A recent study from India unveiled that cancer patients living in rural areas are more likely to die from their disease than patients from metropolitan areas [10]. This finding is very likely characteristic of most LMICs. An important proportion of the burden of cancer could be prevented using the already existing cancer control programmes for tobacco control, vaccination, early detection and treatment programmes, as well as public health campaigns promoting physical activity and healthier dietary patterns [11].

Many obstacles still prevent the effective management of cancer patients in LMICs. The main obstacles include:

- Availability of drugs and treatment facilities
- Cost of drugs
- Distance to dedicated oncology unit
- Compliance with treatment
- Delayed diagnosis and earlier consultation with traditional practitioners
- Lack of follow-up
- Cultural barriers [3, 9]

Nevertheless, one of the biggest challenges for oncologists from LMICs is not just finding treatments for their patients with cancers but finding cancer treatments that could be both affordable and accessible. Thus, to partly reduce the cost of treatments, inexpensive anticancer agents, such as those on the WHO's list of "essential drugs for cancer therapy" [12], should be prioritised. Indeed, most of these agents have generic equivalents, thus paving the way for less expensive treatments.

Still, development of cancer care strategies in LMICs should not be limited to copying and pasting suboptimal, sometimes unrealistic, strategies used in the past in HICs. Instead, we strongly advocate for innovation. Thinking slightly outside of our current standards or deliberately outside the box may allow us to generate new constraint-adapted therapeutic strategies for cancer patients living in LMICs. A number of low-cost and low-technology attempts that could be potentially administered by nonspecialists and have a significant impact on cancer control in developing countries have already been used [9, 13], including metronomics [3].

Elsewhere, to implement innovations for the treatment of cancer patients in LMICs, some lessons can be learnt from the business community in addition to relying on the success stories of certain clinical trials. Such lessons include insights on how to innovate business models in addition to introducing new products, new processes or new management techniques, in particular in healthcare [3].

17.2 Metronomics to Improve Availability of Cancer Treatments

17.2.1 Metronomic Chemotherapy

MC can be defined as the chronic administration of chemotherapy at relatively low, minimally toxic doses on a frequent schedule of administration, with no prolonged drug-free breaks [4, 14]. Initially thought to act by targeting tumour angiogenesis [14], additional mechanisms have been recently unveiled such as its effects on the immune system and cancer stem cells [4] and MC is now considered to represent a form of multi-targeted therapy [3, 15].

As reviewed recently [3], MC has gained increasing and sustained interest in the clinic and showed promising results in phase II clinical studies for the treatment of adult patients with various types of advanced and/or refractory tumours including metastatic prostate and breast cancers. Therefore, several phase III clinical trials are currently underway (www.clinicaltrials.gov) including for the treatment of metastatic (NCT01131195) and triple negative (NCT01112826) breast cancer or advanced colorectal carcinoma (NCT00442637). Several studies are also ongoing in children using combination of chemotherapy agents (NCT00578864) or combination of MC and targeted agents (NCT01517776, NCT00885326). Of note, three randomised trials based on metronomics protocols are also underway in children with rhabdomyosarcoma (NCT00379457) and refractory/relapsed solid tumour post transplantation (NTC 01661400) and for children in palliative care living in India (NTC01858571).

17.2.2 Drug Repositioning

Drug repositioning consists in using "old" drugs for new indications [15, 16]. Testing already established drugs for a potential effect on cancer cells provides several advantages:

- Side effects are known, usually moderate, and have already been well reported.
- Development can be fast tracked since phase I studies are not mandatory, allowing repositioned drugs to potentially enter directly phase II studies to test their anticancer efficacy.
- Cost of drugs is reduced since most of these drugs are available as generics.

Many examples of successful or potential drug repositioning are available in the field of medical oncology [3] with drugs such as celecoxib as an anti-angiogenic

agent [17]; valproic acid as an HDAC inhibitor [18]; statins as multi-targeted agents [19]; metformin as an AMPK/mTOR inhibitor or epithelial–mesenchymal transition inhibitor [20]; itraconazole [21] and/or arsenic trioxide [22] as a sonic hedgehog inhibitor; nifurtimox [23] as an inhibitor of tyrosine-related kinase B; or more recently propranolol as an anti-angiogenic and immuno-modulatory agent [24].

Some of these agents appear to exert anticancer effects against a wide range of malignancies such as metformin and propranolol, while others seem to have a narrower spectrum of activity such as nifurtimox for neuroblastoma and medulloblastoma. Interestingly, repositioned drugs often display new mechanisms of action that could previously be achieved only by using expensive new anticancer agents, therefore providing an effective and affordable alternative to novel targeted therapies for cancer patients.

17.3 Metronomics

While there is currently no clear definition of metronomics, it can be defined as the science associated with metronomic scheduling of anticancer treatment (MSAT), which therefore embraces both MC and drug repositioning [3, 15].

Overall, metronomics displays several practical advantages to help in bringing anticancer treatment opportunities for patients living in LMICs:

- A relatively low direct cost thanks to the use of old and inexpensive generic drugs.
- Easy access to treatment as these drugs are also usually available in oral form avoiding costly hospitalisations and IV injections.
- No requirement for central venous access contributing to decreasing both the cost of treatment and the risk of infection, while increasing feasibility.
- Treatment on an outpatient basis since oral treatments can be taken at home and do not require long fastidious travels to care centres, thus potentially decreasing abandonment of treatment.
- Limited toxicity, thus reducing the risks of secondary infections or additional nutrition problems, and not requiring complex supportive care.
- Multidisciplinary teams that include physicians, pharmacists, nurses and laboratory technicians who must be properly trained to administrate toxic IV chemotherapy are not required.

As a result, MC is easy to administer and does not require complex infrastructure or highly trained human resources. Therefore, light oncology units can easily be introduced, even in rural areas of LMICs where dedicated facilities are frequently lacking.

As we previously reported [3], metronomics is gaining interest from physicians and patients in LMICs. For instance, metronomics protocols that have been used in HICs can also be applied in LMICs for patients with breast or prostate cancer using cyclophosphamide and methotrexate [25, 26]. Similarly, metronomic capecitabine can be used in heavily pretreated patients with metastatic breast cancer [27]. Recently, specific protocols have been set locally for children with advanced or

recurrent disease in Mali [28, 29] and in India [30, 31]. The Indian Journal of Cancer has recently released a special issue focusing on metronomics confirming the interest and the potential of this approach [32].

17.3.1 Frail Patients

In both HICs and LMICs, the use of metronomics for frail patients is also an opportunity to provide treatment to patients who would otherwise not be able to tolerate conventional MTD chemotherapy. For instance, MC has shown very promising results in elderly patients [33]. The risk of cancer increases with age, and as the world population is consistently ageing, the number of elderly patients in need of access to anticancer treatment is bound to drastically increase in the coming years. Toxicities associated with anticancer treatments and potential benefits should be carefully evaluated and weighted eventually calling into questions the relevance of standard MTD chemotherapy. The use of MC has already been reported in elderly patients with breast cancer [34], prostate cancer [35, 36], soft tissue sarcoma [37], gastric cancer [38] and melanoma [39], where it can lead to a control of the disease in a significant proportion of patients.

The presence of organ dysfunction associated with ageing or secondary to cancer itself or other disease can also provide a window of opportunity for the use of MC as illustrated by a trial evaluating a metronomics combination in patients with hepatocarcinoma. Thus, in patients who were not eligible to receive sorafenib, metronomics resulted in long-lasting responses [40] and metronomic capecitabine was reported to be well tolerated by patients with advanced HCC and displayed activity both in treatment-naive patients and in those previously treated with sorafenib [41].

Recently, MC has also been successfully proposed to patients with myeloma with severe heart failure [42, 43]. Thus, in 54 patients receiving a combination of cyclophosphamide and steroids, clinical benefit was achieved in 63 % of patients, including two complete responses. Of note, the left ventricular ejection fraction increased by more than 20 % in 22 out of 34 patients experiencing clinical benefit. Similarly, in previously heavily treated patients who may not be able to tolerate the hematologic toxicity of chemotherapy any longer, MC represents a realistic therapeutic option as already reported in several settings such as multiple myeloma [44].

17.4 Metronomics, New Business Models and Social Values

In the sections below, we will expand on the definition of business model innovation (BMI); explain how it relates to other innovation types such as product, process and management innovation; and provide several examples of BMI inside and outside the field of health and cancer in developed countries to ground our discussion in the actual business practices as well as in LMICs. We argue that in contrast to product or process innovation, BMI can be implemented without important investments into R&D, as it involves the reconfiguration of activities that might be accomplished

using standard technologies easily available on the marketplace. This is particularly important in the context of LMICs where resources are very scarce and the GDP per capita is extremely low. Moreover, BMI enables the creation of virtuous circles where not only the customers (i.e. cancer patients and their family) but also other participants in the business model can reap significant benefits from value creation and can ultimately lead to social innovation.

17.4.1 Business Model Innovation

Interest in innovative business models has sharply increased in recent years. This is because advances in information and communication technologies have enabled companies to fundamentally change the ways they "do business", in particular, the ways they organise activities within the company itself and across industry boundaries with customers, suppliers, partners and other stakeholders. BMI has become an alternative for general managers and entrepreneurs to create value, specifically in times of economic change and turmoil. Business models emphasise a system-level, holistic approach towards explaining how companies conduct business. As such, business models seek not only to explain the ways in which value is captured but also how it is created. Despite the increased interest in new business models, few links have been made between the needs of LMICs in terms of healthcare and the new possibilities opened by BMI. Here, we undertake to show how new business models together with new therapies such as metronomics could contribute to solving some of the long-standing health problems in LMICs.

Several definitions of new business models have been proposed in the literature. Markides speaks of BMI as the search for new ways to create and capture value for the companies' stakeholders by focusing primarily on finding new strategies to generate revenues and define value propositions for customers, suppliers and partners [45]. In addition to differentiating with new or better products, companies thus have a strategic option to compete through different business models. This approach allows for more sophistication than just product innovation (i.e. developing a new anticancer agent). Markides speaks of the discovery of fundamentally different business models in existing businesses [46]: "To qualify as an innovation, the new business model must enlarge the existing economic pie, either by attracting new customers into the market or by encouraging existing customers to consume more." Business model innovators therefore do not necessarily introduce new products or services, but instead redefine what an existing product or service is and how it is delivered to customer. Companies such as Amazon or Swatch are considered as business model innovators because they enlarged their existing markets and attracted new customers to existing products through BMI. For instance, Swatch did not only introduce new products but redefined why a customer would want to buy a watch, thus changing watch attributes from timekeeping to being fashion accessories.

Building on previous BMI definitions, we focus on value creation and activities, two agreed-upon elements present in most business model definitions [47]. The seminal work by Amit and Zott [48] has identified value creation as the overall

objective for a company's business model, and many authors agree that activities can be used as the underpinning analytical element to think about BMI [45, 48–50]. In this vein, we define BMI as *designing* a *new activity system that increases and/or changes the distribution of value created for the participants in the business model, such as customers, suppliers or the focal firm itself.* The focal firm can shape the activity system by directly redesigning its own activities but also by influencing how other actors engage with the firm. The key point here is to focus on activities that create value through resource allocation not only for customers but also for other participants, such as suppliers or other partners.

To summarise, BMI involves the design of a new activity system that (1) affects the total value created through it and (2) the distribution of that value to the different participants in the business model.

The emphasis on BMI encourages systemic and holistic thinking when undertaking innovation. Although BMI can originate within one company, it usually involves reconfiguring the activity system of the whole industry, with repercussions outside the boundaries of the company as it would be the case for the pharmaceutical industry if access to oral anticancer agents was generalised to LMICs. Moreover, BMI by definition creates additional value for several stakeholders, including but not limited to customers, and induces bargaining about value appropriation among those stakeholders [51].

17.5 BMI and Other Innovation Types

Today, companies have a choice between innovating products, processes, management techniques or business models [52–54]. BMI differs from other types of innovation on key dimensions, such as unit, level of analysis and theoretical grounding. Moreover, BMI research operates at a distinct level of analysis, spanning the company and its entire industry [55, 56], while product and process innovation research is mainly centred around the firm [53, 57], and management innovation research compares new management techniques and their implementation across industries and countries [54, 58] taking into account their specificities and needs.

There is a possible interaction between BMI and other types of innovation. Companies can certainly innovate their products without necessarily innovating their business models, which is the case most of the time in the pharmaceutical industry, where the launch of new drugs is rarely combined with BMI. In contrast, when companies innovate their product or service alongside their business model, they can strengthen their brand and create positive spillovers [55]. For instance, when Apple introduced its new product, iPod, embedded within the online music distribution through the iTunes Music Store, it was able to lock its customers in a very successful new business model [59].

Another important advantage of BMI, especially relevant in the context of LMICs, is that it can often be implemented at a relatively low cost, without significant investment into R&D, unlike for product innovation efforts such as

developing new anticancer agents. For instance, eBay implemented its very effective new business model, revolutionising the second-hand goods sale, by investing only in efficient software scripts rather than undertaking any large-scale R&D effort about how to create a perfect market [60]. Moreover, Chesbrough and Rosenbloom illustrate the challenges faced by established companies not considering BMI using the printing company Xerox as an example [61]. Although Xerox was very successful at developing new products, it failed notoriously at profiting from these innovations as Xerox permanently relied on its vertically integrated business model instead of experimenting with more open activity systems like its spin-off companies did. Therefore, one could postulate that pharmaceutical companies that do not commit to BMI and persist in using their traditional business models will have difficulty taking advantage of the emerging market of cancer drugs in LMICs.

17.6 Business Model Innovation in LMICs

As mentioned above, when innovating, companies have a choice between introducing new products, processes or business models. They can also choose among these innovation options when operating in LMICs. For example, Procter & Gamble and Unilever have been introducing cheaper versions of their products in smaller serving sizes for years in countries such as India [62]. The Haier Group in China is known to have introduced washing machines that could be used for multiple tasks by the rural population, including cleaning potatoes or making cheese from goat milk, thus justifying more fully the expense for such a machine in a low-income household [63]. In addition to product innovations, companies can introduce new business models in LMICs, often taking advantage of new technologies that facilitate the structuring of interactions in novel ways. Although several authors mention the potential advantages of BMI in LMICs [54], very few have addressed the question of how exactly companies should innovate in these markets. However, there are many factual examples of companies successfully innovating business models in LMICs. For instance, observing that while most Kenyans have access to a mobile phone but very few have a bank account, M-Pesa has provided access to microfinance services in Kenya, opening the door to easier financing for a population long deprived of such opportunities [64]. Elsewhere, the pharmaceutical company Roche teamed up with Swiss insurance Swiss Re and five local insurers in China to propose a way to sell anticancer drugs to millions of Chinese patients who couldn't otherwise afford them by first selling them insurance. This strategy allows creating a new market by challenging the erroneous conception that there are only two segments in LMICs: the rich, who can afford it, and the poor who cannot afford it at all. In another example of BMI in LMICs, the Grameen Group in Bangladesh has established partnerships with companies such as Norwegian Telenor to sell mobile services in rural villages and French Veolia to distribute drinkable water through simplified water plants combining both a social and a profit motive in new business models [65].

It is worthwhile to observe that BMI can be usefully conducted not only by creating value for customers but also by increasing the value for other stakeholders involved in the activity system, thus establishing virtuous circles of value creation in these markets [66].

Aravind presents another fascinating example of BMI in the healthcare sector in India. Founded in 1976 by eye surgeon, Dr. Venkataswamy, Aravind Eye Hospital embarked on the mission to eliminate preventable blindness. The organisation has become very successful, converting itself into the largest provider of eye care in the world and acting as an example for other hospitals in LMICs as well as developed countries [67, 68]. Aravind innovated by providing eye surgery for its patients and by extending into manufacturing of intraocular lenses that otherwise were too expensive on the market for its low-income patients to afford. Aravind squarely focused on lowering costs in all activities of its business model by leveraging high volumes of patients as a driving force for learning curves and economies of scale. For example, working alternatively on two operating tables, Aravind surgeons can perform between six and eight surgeries per hour [66]. Moreover, Aravind created a system where patients that can afford to pay more for eye surgeries cross-subsidise patients that cannot afford such surgeries, while providing the same level of service to all patients involved [68]. By developing such a new business model, Aravind has been able to advance towards its goal of eliminating preventable blindness in India. Several lessons can be learnt from this BMI example in LMICs – high volume of patients enabled Aravind to create a virtuous circle, lowering their fixed costs and increasing their bargaining power with various suppliers, but also becoming the preferred training ground for highly qualified surgeons, leading to long-term and sustainable growth.

17.7 Metronomics and New Business Models

Based on the principles and examples mentioned above, we truly believe that metronomics therapy needs to be combined with new business models to successfully contribute to solving the problem of cancer treatment in LMICs.

Many actions can be undertaken such as increasing healthcare coverage, relying as much as possible on generic drugs and biosimilars, eventually introducing earlier generic drug competition. Participation in clinical trials should also be encouraged to demonstrate the benefit of treatments in LMIC setting, and funding of these studies might be fuelled by innovative incentives directed to the pharmaceutical industry. For instance, we recently advocated for the creation of powerful incentives, in line with those set for paediatric treatments in HICs such as offers of patent extension, through new business models and strategic partnerships to help the development and accessibility of treatments for patients with cancer living in LMICs [59]. Additionally, new payment methods could be introduced like price discrimination, access programmes or risk-sharing agreements according to which no payment is required for patients who do not benefit from the treatment. Lastly, cooperation among stakeholders like public–private partnerships and philanthropy must be initiated [1].

From existing research on BMI, we know that it is usually new, rather than established firms that introduce new business models [56, 69]. We can thus expect that new companies or non-profit organisations might be at the origin of introducing metronomics to LMICs rather than traditional pharmaceutical companies. However, the participation of bigger players, such as established pharmaceutical companies, might be critical to successfully solve the large-scale health issues in LMICs. Thus, a model of cooperation between new companies with innovative ideas, but usually lacking resources, and existing pharmaceutical firms, lacking flexibility, but possessing important financial, distribution and marketing resources, could provide a potential solution. Additionally, government involvement might also be required in specific countries to facilitate the overall process as well as to reach rural populations.

The challenges of metronomics remain numerous, such as treatment price, patient acceptance and compliance, distribution in rural areas, government regulations, financing of the whole system of care, access to resources and capabilities for the local businesses and their employees, as well as overcoming barriers to the actual formation of partnerships with existing players in the pharmaceutical industry. On the other hand, the advantages of implementing metronomics accompanied by new business models include the low reliance of BMI on R&D investment, as well as the development of virtuous circles of value creation between various stakeholders involved [70]. Although difficult to design, new business models can bring a significant relief to LMIC cancer patients, following the example of the Aravind eye care model in India.

One of the biggest challenges remains funding treatments that are usually too expensive for the average patient to afford. Payment systems in healthcare greatly vary around the world, with some developed countries achieving universal healthcare access through a mixture of public and private funding. However, general taxation revenues are low to non-existent in LMICs, and very few healthcare services are covered for the poorest. BMI thinking could be very helpful in this case to imagine innovative solutions to finance cancer treatment in this context. Aravind, for instance, cross-subsidises groups of patients, where richer patients pay a fee for their treatment, and the poor patients receive fee-free surgery. The equality of such a system has to be carefully monitored to ensure all patients perceive being treated with justice, and it can only be achieved if paying patients know that they receive the best care available. Other options, such as crowdfunding on Internet platforms such as Kickstarter or Kiva, relying on NGOs or partnering with established pharmaceutical companies, might be considered to design the most pertinent business models in given contexts. Such collaboration has been successfully proposed by *the Learning Collaborative* [71]. Indeed, this initiative was launched by leaders from the industry, governments, academics and non-profit organisations, in order to promote drug repositioning for the treatment of cancer patients by building a new academic model and working on regulatory and public policies. While the target population lives in HICs, this is an example of an initiative where non-traditional, dynamic partnerships allow non-profit organisations to play a major role in advancing the development of new anticancer therapies and bringing these therapies rapidly to patients.

Conclusion

The cancer burden will increase significantly in both HICs and LMICs in the next 10 years. In LMICs, it will result in an increase in cancer deaths occurring as a direct consequence of resource limitations. Although there is a growing awareness of the cancer problem in LMICs and despite the development of global oncology, concrete innovative proposals to address this issue are still too rare and the main challenge is to propose affordable, accessible, safe and effective treatments for cancer patients living in these countries. To treat cancer for a dollar a day [13], beyond medical and scientific advances, we believe stakeholders should also focus on BMI and cooperate to develop sustainable and socially responsible anticancer strategies.

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Adverse Side Effects Associated with the Use of Low-Dose Metronomic Chemotherapy

18

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Abstract

The benefit of cancer therapies can be characterized by the therapeutic index incorporating the balance between antitumor activities and treatment-associated toxicities. Low-dose metronomic (LDM) chemotherapy is a novel treatment strategy that was developed to overcome resistance to maximum-tolerated dose (MTD) chemotherapy, while using conventional chemotherapeutics. Published findings suggest that the therapeutic index of LDM chemotherapy is particularly beneficial given the combination of excellent antitumor activity with a toxicity profile that is considered to be superior to MTD chemotherapy. In fact, a systematic analysis of 66 published phase I/II clinical LDM chemotherapy trials providing detailed toxicity data confirms the excellent safety profile of LDM chemotherapy. Severe (grade 3 or 4) side effects were rare, and the toxicity profiles seemed to be associated with the type of LDM regimen studied. Overall, incidences of any severe adverse effects were limited to less than 10 % of patients. Severe lymphopenia and neutropenia were the most frequent side effects reported, occurring in 6.44 % and 5.71 % of patients. Furthermore, three

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comparative studies reporting the rates of adverse events associated with LDM versus MTD regimens indicate superior tolerance and safety of LDM chemotherapy, while still displaying comparable antitumor effects. By making treatments more tolerable and thus accessible to a broader range of cancer patients, LDM chemotherapy is an example that less can sometimes be more. However, in the absence of validated predictive or pharmacodynamic markers of LDM chemotherapy, additional clinical studies are warranted to further improve the therapeutic index of LDM chemotherapy.

18.1 Introduction

The overall benefit of cancer therapy depends on the therapeutic index, a balance between the antitumor activities achieved and the level of treatment-associated toxicities. While the relatively high rate of acute side effects associated with conventional, maximum-tolerated dose (MTD) chemotherapy is acceptable in the (neo) adjuvant setting due to a possible curative prospect, the balance shifts towards minimizing harm and maintaining comfort during later stages of incurable disease.

Low-dose metronomic (LDM) chemotherapy aims at improving both aspects that define the therapeutic index. This novel use of conventional chemotherapeutics was developed to overcome resistance to MTD chemotherapy by shifting the primary treatment target from the highly adaptive tumor cell population to the more stable tumor vasculature [1, 2], and it is based on a number of key findings [3, 4]. Tumor endothelial cells are usually highly proliferative, which may explain, among others, their exquisite sensitivity to conventional chemotherapeutics [5, 6]. On the other hand, tumor endothelial cells also possess advanced repair capabilities that may be enhanced by a variety of circulating bone-marrow-derived cells, including endothelial progenitor cells [7]. Such rapid and marked vascular repair is seen with MTD chemotherapy, which commonly results in rapid and robust mobilization of bone-marrow-derived cells. In addition, MTD chemotherapy has an additional disadvantage from an antiangiogenic perspective, i.e., the need for treatment-free intervals to enable recovery from acute side effects such as myelosuppression. In contrast, the LDM way of chemotherapy administration emphasizes antivascular effects by virtue of minimizing the need for treatment-free intervals and by diminishing repair processes mediated by bone-marrow-derived cells. Since potent antiangiogenic effects can be achieved with relatively low doses of conventional chemotherapeutics, a welcome "side effect" of LDM chemotherapy is the paucity of severe acute treatment-associated adverse effects. As such, LDM chemotherapy is an interesting treatment alternative, especially for palliative indications and for the elderly and/or frail patients that otherwise would not be candidates for MTD chemotherapy [8, 9].

In the absence of suitable predictive or pharmacodynamic markers [10], clinical applications of LDM chemotherapy remain largely empirical when it comes to the choice of a given chemotherapeutic, the dosing (often flat dosing), and the administration schedule (defined primarily by practical considerations, e.g., availability of

oral formulations and their dosages). On the other hand, all these factors may be of substance when it comes to antiangiogenic and other effects of LDM chemotherapy, including immunomodulation [11]. In fact, it remains to be seen if LDM regimens can be optimized for a given patient regarding their beneficial activities (i.e., antiangiogenic effects, immunostimulation) while at the same time minimizing negative aspects, such as myelosuppression, immunosuppression, and other adverse side effects. Collectively, clinical trial data seems to confirm the excellent tolerance of LDM chemotherapy [4, 12–15]. Herein, we summarize preclinical and clinical aspects of adverse side effects associated with LDM chemotherapy.

18.2 Preclinical Toxicity Studies

A detailed comparative toxicity analysis of LDM versus MTD cyclophosphamide therapy in mice revealed the absence of relevant myelosuppression and gastrointestinal toxicity of LDM cyclophosphamide (20 mg po daily via drinking water) despite significant anti-CD31 microvessel activity [16]. This study also suggested that the tumor microvasculature is more sensitive to LDM cyclophosphamide than wound healing-associated angiogenesis. On the other hand, the same LDM cyclophosphamide regimen resulted in sustained lymphopenia, a finding that has been confirmed in clinical trials using metronomic cyclophosphamide [17]. Thus, for some agents and LDM regimens, there may be an overlap between the dose range leading to desirable antiangiogenic effects and unwanted severe lymphopenia.

Shaked et al. described a preclinical method to determine the optimal LDM dose of a given chemotherapeutic agent based on minimal myelosuppressive activity, associated with maximal reduction of circulating endothelial progenitor cells [18]. The optimal biological LDM dose determined in this way was associated with maximal antitumor effects in a panel of tumor models subjected to chemotherapeutics with different mechanisms of action. In other words, there appears to be a dose range of maximal antitumor and antivascular activity that is associated with minimal toxicity. However, the potential negative impact of lymphopenia on the antitumor effects of LDM chemotherapy could not be addressed definitively in three of the four studies that involved the transplantation of human xenografts into immunodeficient mice. In addition, the proposed method is of limited clinical utility for a number of reasons, including methodological challenges to reliably determine circulating endothelial progenitor cells in humans [19] and practical hurdles that would be associated with intrapatient dose escalation (especially in complex treatment regimens comprising more than one drug).

Kamat et al. analyzed different dose levels of thrice a week docetaxel LDM therapy [20]. Their study confirmed that maximal antitumor activity could be achieved without overt toxicity (i.e., weight loss). In addition, they found an LDM docetaxel threshold dose similar to what had been predicted by mathematical modeling [21].

Overall, preclinical evidence suggests that robust antitumor effects can be seen without significant associated toxicity. On the other hand, we recently observed severe gastrointestinal stasis in mice treated with the optimal biological dose of

docetaxel, as determined by the method of Shaked et al. [18, 22]. Furthermore, complex u-shaped or bell-shaped dose-response relationships have been described in the context of LDM or other antiangiogenic therapies [23, 24].

18.3 Toxicity-Guided Clinical Dose-Finding Studies

There are only few dose-finding LDM studies in humans that were oriented towards minimizing adverse events and optimizing uninterrupted treatment administration. Takakashi et al. determined the individualized maximum repeatable dose of weekly gemcitabine by weekly adjustments of the gemcitabine dose to prevent grade 2 or higher side effects [25], whereas Briasoulis et al. applied a modified dose-escalation strategy to define the highest tolerated dose of oral vinorelbine administered thrice a week [26]. In the latter study, toxicity was deemed unacceptable in cases of any grade 4 event or if grade 2 or 3 adverse events would result in treatment discontinuation for more than 2 weeks during the first 2 months of treatment.

In contrast to dose-finding studies, there is a lack of preclinical or clinical studies to address the impact of dosing frequency. Mathematical modeling suggests that continuous dosing would be ideal to sustain drug levels just above the 'antiangiogenic' LDM threshold dose [21]. In practical terms this can be best achieved with oral agents such as cyclophosphamide given on a daily basis. On the other hand, continuous dosing is not necessarily identical with LDM chemotherapy. As an example, in the 1980s and early 1990s, prostate cancer chemotherapy consisted mainly of metronomic-like oral regimens of cyclophosphamide, etoposide, or estramustine [27, 28]. However, the dosing of such regimens was oriented towards MTD chemotherapy administration, and thus planned treatment interruptions were common [29]. In fact, frequent dosing can be used to increase the cumulatively administered dose of drugs such as temozolomide [30]. The dosing frequency may also change the type and incidence of side effects seen, as evidenced, for instance, by comparing the toxicity profiles of docetaxel chemotherapy for castration-resistant prostate cancer given either weekly or every 3 weeks [31] or infusional versus bolus 5-FU (fluorouracil) administration [32]. With respect to 5-FU, the administration mode seems to also affect the mechanisms of antitumor activity.

18.4 Overview of Toxicities Encountered in LDM Chemotherapy Trials

A recent systematic review of 80 published phase I/II LDM chemotherapy trials encompassing 3,688 patients (only studies with at least 20 patients were included), varying cancer types and different LDM regimens reported overall low toxicity rates [12]. Neutropenia was the most common severe (i.e., grade 3 or 4) side effect affecting 5.39 % of all patients. Deaths considered as being associated with LDM therapy were reported in 0.4 % of patients. Of the 80 published trials, we focus herein on 66 phase I and II clinical trials, the reports of which provide detailed

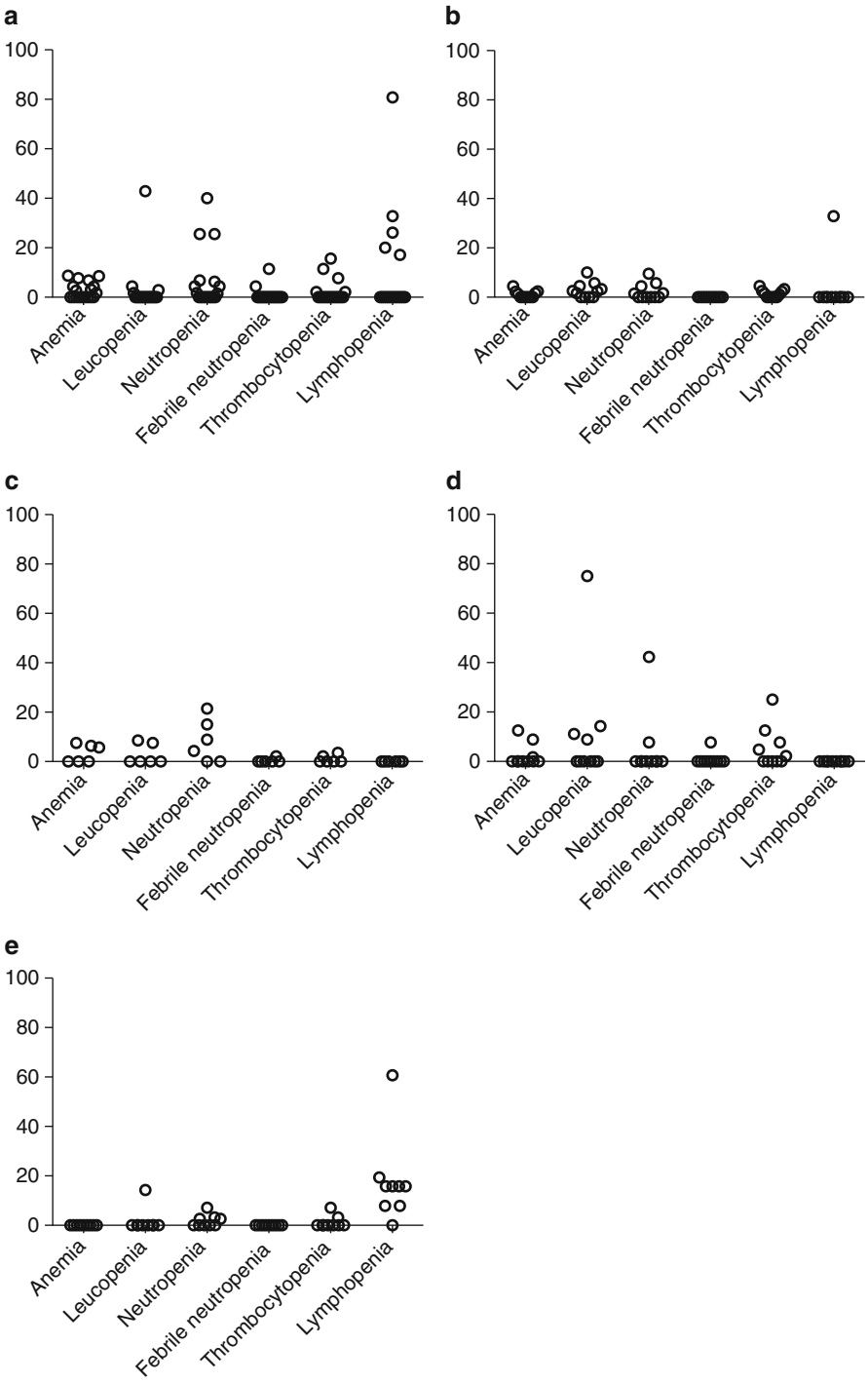
Table 18.1 Rates of severe (grade 3/4) side effects associated with low-dose metronomic chemotherapy

Grade 3 or 4 (severe) side effects	Number of patients (%) $N=2,920$
Hematological	
Lymphopenia	188 (6.44)
Neutropenia	167 (5.71)
Leucopenia	119 (4.08)
Anemia	65 (2.22)
Thrombocytopenia	56 (1.92)
Febrile neutropenia	20 (0.67)
Non-hematological	
Elevated transaminases	88 (3.00)
Fatigue	78 (2.66)
Hand–foot syndrome	34 (1.16)
Thrombosis	24 (0.83)
Colitis/mucositis/stomatitis	24 (0.82)
Nausea/vomiting	22 (0.76)
Infection	19 (0.66)
Diarrhea	15 (0.51)
Neurological	7 (0.23)

toxicity results ($n=2,920$ patients), and on severe side effects observed. Among these studies, 55 (83 %) reported severe hematological side effects [8, 17, 26, 33–87], and 53 (80 %) reported severe non-hematological side effects [8, 26, 33–49, 53–58, 63, 65, 67, 70, 71, 74–78, 80–84, 86, 88–95]. Overall, lymphopenia and neutropenia were the most frequent adverse events observed in the pooled patient population, occurring in 6.44 and 5.71 % of patients (Table 18.1). Severe hematological side effects were more frequently observed than severe non-hematological toxicities, which affected less than 3 % of patients for any given adverse event. The side effect profiles appear to be more profoundly affected by the type of LDM regimen than the type of tumor studied. This is true for both hematological (Fig. 18.1) and non-hematological toxicities (Fig. 18.2).

18.5 Side Effects Associated with Specific LDM Chemotherapy Regimens

Among the 66 publications reporting detailed information on treatment-associated side effects, the following frequently used regimens were identified: (1) cyclophosphamide-based regimens ($n=16$ studies), (2) cyclophosphamide plus methotrexate regimens ($n=9$ studies), (3) capecitabine-based regimens ($n=6$ studies), (4) etoposide-based regimens ($n=5$ studies), and (4) temozolomide-based regimens ($n=5$ studies). For the purpose of our analyses and the data presented in Fig. 18.1, we disregarded the multitude of concurrent medications administered with the LDM chemotherapy "backbone."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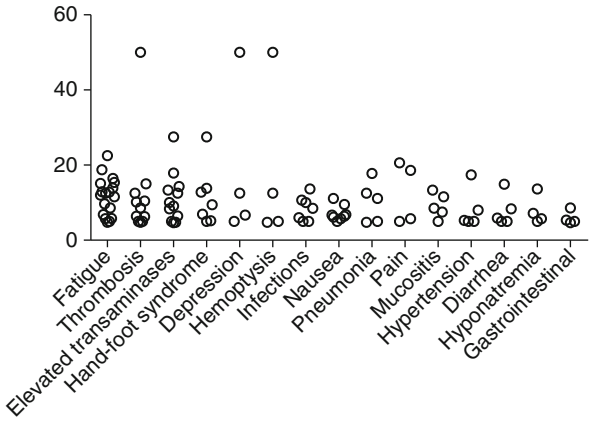

Fig. 18.2 Rates of severe (grade 3/4) non-hematological side effects occurring in ≥5 % of patients ($n=66$ clinical studies)

18.5.1 Cyclophosphamide-Based Regimens

Sixteen trials were identified to use oral cyclophosphamide, administered with other drugs, including other chemotherapeutics (such as capecitabine and vinblastine), letrozole, cyclooxygenase 2 inhibitors (celecoxib and rofecoxib), corticosteroids (i.e., prednisolone, prednisone, or dexamethasone), bevacizumab, and oncolytic adenovirus [8, 17, 36, 45–47, 49, 61, 62, 64, 76, 81, 82, 87, 91, 93]. With few exceptions, most studies used an oral cyclophosphamide flat dose of 50 mg. Lord et al. administered 50 mg/m^2, corresponding to 90 mg daily, assuming an average body surface area of 1.8 m^2 [17, 96], while Mir et al. prescribed cyclophosphamide at 100 mg daily [64]. Collectively, neutropenia and anemia were the most frequently reported side effects (Fig. 18.1a), reflecting the known myelosuppressive potential of cyclophosphamide and of some of the concurrently administered agents. Of note, the rate of severe lymphopenias increases with daily doses of greater than 50 mg. However, such LDM cyclophosphamide-associated lymphopenias did not result in atypical infections. The most commonly reported non-hematological adverse events seen with LDM cyclophosphamide therapy included skin rash, fatigue, nausea, and hypertension.

18.5.2 Cyclophosphamide Plus Methotrexate

Nine published trials reported on LDM cyclophosphamide combined with methotrexate [40, 41, 50–52, 58, 68, 74, 95], a regimen that is particularly popular for the

Fig. 18.1 Rates (%) of severe (grade 3/4) hematological side effects reported in individual clinical studies of common low-dose metronomic chemotherapy regimens. (**a**) Cyclophosphamide-based ($n=16$ studies). (**b**) Cyclophosphamide plus methotrexate ($n=9$ studies). (**c**) Capecitabine-based ($n=6$ studies). (**d**) Etoposide-based ($n=5$ studies)*. (**e**) Temozolomide-based ($n=5$ studies)*. *Note*: *Some studies contain multiple treatment arms

treatment of breast cancer (7 of the 9 studies). With few exceptions, the dose administered for cyclophosphamide was 50 mg daily and for methotrexate 2.5 mg po twice daily on days 1 and 2 of each week. Concurrent therapies included thalidomide, bevacizumab, trastuzumab, celecoxib, prednisone, and/or dalteparin. Overall, the rate of severe hematological side effects was well below 10 % in the majority of studies (Fig. 18.1b). Moreover, no incidence of febrile neutropenia was observed. Only one study reported cases of severe lymphopenia, although the daily dose of cyclophosphamide did not exceed 50 mg [58]. Elevated transaminases, a known side effect of methotrexate administration, were the most prevalent non-hematological side effect. In some patients, transaminase elevation may also have been associated with liver metastases.

18.5.3 Capecitabine-Based Regimens

Six studies which administered capecitabine in combination with other drugs (docetaxel, gemcitabine, celecoxib, sorafenib) were identified [34, 54, 79, 80, 86, 94]. The daily doses of capecitabine varied from 500 mg flat-dosed twice daily to 2,250 mg once daily (1,250 mg/m^2), assuming an average body surface of 1.8 m^2 [96]. The most frequently observed severe hematological side effects were neutropenia, reported in 4 trials [34, 54, 79, 86], and anemia, reported in 3 trials (Fig. 18.1c) [34, 80, 86]. There was only one study that observed an incidence of febrile neutropenia (2.13 %) [86]. Similarly, incidences of grade 3 or 4 lymphopenia were absent. In contrast to the aforementioned LDM regimens, cases of severe non-hematological side effects were reported with a higher frequency, including fatigue, hand–foot syndrome, stomatitis, elevated transaminases, and diarrhea. With the exception of fatigue, the aforementioned are adverse effects commonly attributed to the use of 5-FU analogues, such as capecitabine. Of note, some of the LDM regimens applied capecitabine doses similar to what is considered to be conventional MTD dosing.

18.5.4 Etoposide-Based Regimens

Five studies prescribed daily oral etoposide, with a dosage ranging from 50 to 100 mg daily, and co-medications such as bevacizumab and cisplatinum [42, 44, 56, 71, 72]. Overall, LDM etoposide had a myelosuppressive profile comparable to cyclophosphamide (Fig. 18.1d). Despite this predilection, no incidence of severe lymphopenia was observed. On the other hand, LDM etoposide was associated with numerous severe non-hematological toxicities, which included renal failure, hemoptysis, thromboembolic complications, mucositis, and fatigue/asthenia.

18.5.5 Temozolomide-Based Regimens

Four studies administered temozolomide monotherapy with doses of 40–75 mg/m^2, and one trial administered temozolomide combined with bevacizumab [37, 59, 60,

70, 71]. Four of the five studies analyzed the effects of temozolomide-based regimens on patients diagnosed with glioblastoma [37, 59, 70, 71], and one examined temozolomide monotherapy on lung carcinoma patients [60]. While no incidences of either grade 3 and 4 anemia or febrile neutropenia were reported, four studies documented cases of severe lymphopenia [37, 59, 60, 70], and three reported on severe neutropenia [37, 59, 60] (Fig. 18.1e). In addition, severe fatigue was a common non-hematological event associated with LDM temozolomide therapy.

18.6 Comparative Analyses of LDM Versus MTD Chemotherapy-Associated Side Effects

While the low rate of severe side effects described in LDM chemotherapy trials suggests that LDM chemotherapy is better tolerated than MTD treatment, only few trials have studied an LDM versus an MTD treatment arm in parallel (Table 18.2).

Vorob'ev et al. reported a nonrandomized phase II study comparing the efficacy and safety of LDM cyclophosphamide (50 mg po daily) in 25 patients with castration-resistant prostate cancer and 30 patients from the same institution that had received MTD docetaxel therapy (75 mg/m^2, every 3 weeks) [97]. While there was no significant difference in the median survival time between the LDM and MTD treatment options (15.4 ± 2.2 versus 15.9 ± 1.7 months, respectively), some of the secondary endpoints (PSA response rate, pain, and quality of life assessment) appeared to favor the MTD arm numerically, albeit not in a statistically significant manner. Mild cases of anemia (grades 1 and 2) were the most frequent side effect observed, with an incidence of 80 and 40 % in the MTD and LDM arms, respectively. However, no severe adverse events were reported for the LDM cyclophosphamide arm. As expected, 20 % of patients undergoing conventional docetaxel chemotherapy progressed to grade 3 or 4 anemia. In addition, grade 3 or 4 neutropenia was documented in 13 % of the patients receiving docetaxel.

Clarke et al. examined the benefit of dose-dense temozolomide chemotherapy (150 mg/m^2 on days 1–7 and days 15–21 of each 28 day cycle) versus LDM temozolomide (50 mg/m^2 continuously) in patients with newly diagnosed glioblastoma multiforme, following radiation therapy with concurrent daily temozolomide given at 75 mg/m^2 [37]. 42 patients were randomized to the dose-dense arm and 43 patients to the LDM arm. The median overall survival was comparable (17.1 and 15.1 months, respectively). Generally, few side effects (mild or severe) were reported, with higher incidences occurring in the dose-dense arm. As an example, there were higher rates of myelosuppression and fatigue in the dose-dense temozolomide arm, with two patients removed from this regimen due to persistent or recurrent myelosuppression, despite growth factor support. Of note, there was a higher incidence of elevated transaminases in the LDM treatment arm (18 % versus 3 %). Severe lymphopenia had the greatest rate of occurrence, with no significant difference between the two regimens (68 % and 61 %, respectively, for dose-dense and LDM arms).

In the *C*hemotherapy *A*djuvant *S*tudy for women at advanced *A*ge (CASA) trial, Crivellari et al. attempted to study LDM cyclophosphamide and methotrexate

Table 18.2 Severe (grade 3 and/or 4) side effects in MTD versus LDM treatment arms arising in the studies by Vorob'ev et al. [97], Clarke et al. [37], and Crivellari et al. [98]

	Vorob'ev 2011 (%)		Clarke 2009 (%)		Crivellari 2013 (%)	
	MTD ($n=30$)	LDM ($n=25$)	MTD ($n=31$)	LDM ($n=28$)	MTD ($n=37$)	LDM ($n=35$)
	TAX 75 mg/m^2 iv q3 weeks	CPA 50 mg po od continuously	TMZ 150 mg/m^2 2/4 weeks	TMZ 50 mg/m^2 od continuously	PLD 20 mg/m^2 iv q2 weeks	CPA 50 mg po od+MTX 2.5 mg po bid [2/7 days]
Hematological						
Anemia [G3/4]	20	0	–	–	–	–
Neutropenia [G3/4]	13	0	10	7	–	–
Leukopenia [G3/4]	0	0	19	14	–	–
Thrombocytopenia [G3/4]	0	0	3	7	–	–
Lymphopenia [G3/4]	0	0	68	61	–	–
Non-hematological						
Elevated transaminases [G3/4]	–	–	3	18	–	–
Fatigue [G3/4]	–	–	10	4	2.7	0
Constipation [G3]	–	–	–	–	2.7	0
Mucositis/stomatitis [G3]	–	–	–	–	5.4	0
Infection [G3]	–	–	–	–	8.1	0
Rash, hand–foot skin reaction [G3]	–	–	–	–	21.6	0
Other rash [G3]	–	–	–	–	5.4	0
Hypertension [G3]	–	–	–	–	10.8	20
Neurological [G3]	–	–	–	–	0	2.9
Femur fracture [G3]	–	–	–	–	2.7	0
Pain [G3]	–	–	–	–	5.4	2.9
Allergic reaction/ hypersensitivity [G3]	–	–	–	–	5.4	0
Undefined [G3]	–	–	–	–	0	11.43

Abbreviations: '–' data not available/reported, *bid* twice daily, *CPA* cyclophosphamide, *G* grade, *iv* intravenously, *LDM* low-dose metronomic, *MTD* maximum-tolerated dose, *MTX* methotrexate, *od* daily, *PLD* pegylated liposomal doxorubicin, *po* oral, *TAX* docetaxel, *TMZ* temozolomide

chemotherapy in elderly (>65 years) patients with operable, endocrine nonresponsive (ER < 10 % and PR < 10 %) breast cancer presenting with comorbidities precluding the use of standard MTD chemotherapy [98]. Patients were either randomized to (1) 16 weeks of LDM cyclophosphamide (50 mg po daily) and methotrexate (2.5 mg po twice daily, on the 1st and 4th days of each week) versus pegylated liposomal doxorubicin (PLD, 20 mg/m^2 iv every 2 weeks, an "MTD" regimen thought to be better tolerated than classical adjuvant breast cancer regimens) [CASA-CM] or to (2) PLD versus no treatment [CASA-nil]. However, this multinational, phase III randomized clinical trial closed early due to poor accrual. Recently, Crivellari et al. reported data on 37 patients accrued for the LDM arm compared with 38 patients in the PLD arm. After a median follow-up of 42 months and an event in 19 % of all patients, the Kaplan–Meier estimate for the breast cancer-free interval at 3 years was the same in both treatment arms (i.e., 0.78). Patients in the LDM arm reported better quality of life (except for nausea/vomiting) as well as better cognitive and physical functioning than those receiving the PLD regimen. Side effects of any grade were uncommon and rarely exceeded occurrences of >10 %. Hypertension was the only severe side effect affecting at least two patients, which was more common in the LDM arm (7 patients). Unsurprisingly, skin alterations were more common in patients treated with PLD (8 patients).

18.7 Discussion and Outlook

Collectively, the data presented herein and recently published review articles support the notion that LDM chemotherapy regimens are generally very well tolerated [12–15]. The few comparative studies indicate superior tolerance of LDM versus MTD chemotherapy. It remains to be seen if this also applies to LDM chemotherapy in comparison to targeted anticancer agents, which are increasingly used for the treatment of various tumor types. Although there is no definite clinical data, preclinical studies suggest that the optimal biological LDM dose in terms of anticancer activity appears to coincide with very limited toxicity. This would indicate that the lack of severe acute toxicity could be used to optimize LDM chemotherapy dosing in individual patients as suggested by Takakashi et al. [25], but more clinical studies are needed. Of note, preclinical in vivo studies mainly used human tumor xenograft models and thus ignored the potential immunomodulatory aspects of LDM antitumor activity, which are commonly assumed to be dose dependent in nature.

In our analyses, we have focused on severe adverse events since many of the low-grade side effects (i.e., grade 1 or 2) reported in LDM trials may be related to the underlying neoplastic condition rather than to LDM therapy itself. On the other hand, low-grade symptoms could negatively impact adherence to treatment itself, particularly to oral regimens administered over prolonged periods of time. In a phase III trial of metronomic-like UFT (uracil–tegafur, a 5-FU precursor) used for early-stage lung cancer, treatment compliance decreased gradually from 80 % at 6 months, to 74 % at 12 months, to 69 % at 18 months, to 61 % at 24 months [99]. Although grade 3 side effects were exceedingly rare and grade 4 side effects were absent, around 60 % of

patients that opted to stop treatment did this because of supposed UFT-related side effects. In the aforementioned adjuvant breast cancer study by Crivellari et al., 83 % of patients completed 16 weeks of LDM cyclophosphamide and methotrexate chemotherapy [98]. It remains to be seen if attrition rates are similar in patients with metastatic disease. In addition, LDM regimens often comprise other agents that could also negatively impact the side effect profile and thus treatment adherence.

Since LDM chemotherapy is primarily being studied in advanced disease stages, LDM drug administration is often limited to a few months. Furthermore, the overall prognoses of such patients are poor, which could explain why side effects associated with cumulative doses of chemotherapeutics are very rarely reported. As an example, only 1 out of 471 patients with castration-resistant prostate cancer undergoing cyclophosphamide-based LDM chemotherapy developed hemorrhagic cystitis [100]. The use of cyclophosphamide has also been associated with secondary malignancies such as leukemias and urothelial carcinomas. In fact, Dobi et al. recently reported a prostate cancer patient treated with LDM cyclophosphamide (50 mg daily for 36 months) who eventually developed acute myelogenous leukemia with cytogenetic abnormalities frequently observed in alkylating agent-induced leukemias [101]. A cumulative cyclophosphamide dose of >10 g/m^2 (approximately equivalent to 200 days of treatment with 50 mg of cyclophosphamide po daily in a patient with a body surface area of 1.8 m^2) is considered to increase the leukemia risk [102, 103], while the risk of urothelial cancer doubles for every incremental 10 g of cyclophosphamide administered [104]. Furthermore, aside from the cumulative cyclophosphamide dose, treatment durations of more than 1 year seem to be associated with an eightfold increased risk of urothelial cancer. Secondary leukemias have also been shown to complicate continuous etoposide therapy, especially in children and young adults [105].

Despite the recent emergence of numerous targeted therapies, chemotherapy is expected to continue as a major anticancer treatment modality in the foreseeable future. While a number of chemotherapy dose intensification strategies have either failed or resulted in only incremental benefits, they were all associated with increased toxicity [106]. Therefore, by making treatments more tolerable and thus accessible to a broader range of cancer patients, LDM chemotherapy is an example that less can sometimes be more.

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Part IV

Metronomic Chemotherapy in Veterinarian Clinic

Clinical Studies of Metronomic Chemotherapy in Dogs

19

Veronica Marchetti and Mario Giorgi

Abstract

In the last decade metronomic chemotherapy has received increased interest in veterinary oncology. Indeed, low-dose metronomic chemotherapy has been shown an important stabilizing effect on cancer growth, conferring both prolonged clinical benefits and positive effects on the quality of life of patients. A number of studies have been performed in dogs on the efficacy of metronomic dosing of various chemotherapeutic drugs. Metronomic chemotherapy is offered as the treatment of choice for all pets with malignant tumors where owners are reluctant to embark on an aggressive therapy protocol. It is indicated in patients with organ failure in which the toxicity of chemotherapy may be fatal as well as in patients with an aggressive nature that would require sedation for each parenteral administration. Metronomic therapy induces minimal impact on the animal; it is a low-cost alternative and it is easy to administer. Moreover, it has been recently recognized that dogs affected by natural cancer serve as unique animal model for human tumors. For this reason, the metronomic chemotherapy experience in dogs could lead to innovative and unexplored schedules for humans. It may be used as a more accurate model than rodents with induced cancers for the extrapolation of dose, efficacy and safety profiles to humans.

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19.1 Introduction

Similar to human medicine, chemotherapy drugs in veterinary medicine are usually administered at doses that are high enough to result in an obligatory break period to allow for the observation of potential side effects and institution of supportive care, if required. In the last decade, efforts to administer chemotherapy on a more continuous basis, with a much shorter break period, or none at all, have received increased interest, and the practice has come to be known as metronomic chemotherapy. The basis for success with this currently investigational approach may be rooted in continuous drug exposure to susceptible cancer cells, inhibition of tumor blood vessel growth – a process known as tumor angiogenesis – and/or alterations in tumor immunology.

It has been observed that metronomic chemotherapy does not have a significant direct cytotoxic effect against tumor cells but rather is able to modify the tumor microenvironment. One of its prime effects is to delay or render ineffective the tumor's capacity to generate new blood vessels for the tumor stroma. It does this in various ways [1–4]: (1) a selective induction of apoptosis and inhibition of proliferation and migration of activated endothelial cells [5–7], (2) modulating inhibitory (e.g., thrombospondin-1) and stimulatory factors of angiogenesis [7–10], (3) inhibition of endothelial cell microtubules in vitro [11], (4) targeting circulating endothelial progenitor cells (CEPC) [11, 12], (5) modulating the cell cycle through the upregulation of caveolin-1 and the downregulation of cyclin D1 [13], and (6) reducing Treg cells that inhibit cell-mediated immunity against self cells [14] and stimulating the action of dendritic cells [15, 16].

Several studies in veterinary medicine have been designed to demonstrate increased proangiogenic factors during tumor expression. Immunohistochemistry or real-time polymerase chain reaction (RT-PCR) has been used to observe expression of VEGF and/or their receptors and/or MMP in canine breast cancer [17–21], mast cell tumors [22–24], hemangiosarcoma [25], intracranial tumors of various origins [26–28], nasal epithelial neoplasia [29], lymphomas [30–32], melanoma [33], meningiomas [34], squamous cell carcinomas [35, 36], and soft tissue sarcomas [37].

Other studies have tried to correlate this expression with microvascular density (MVD) [31, 32, 37], with expression of COX-2 and MVD [20], and with other factors [19, 25, 34].

Other surveys have focused on observation of blood concentrations of VEGF and/or MMP in various malignancies [38–40] or in well-defined tumors such as lymphoma [41–43], intracranial cancer [28], melanoma [33], hemangiosarcoma [44], breast cancer [45], osteosarcoma [46], and soft tissue sarcoma [47].

Metronomic chemotherapy has been shown to have an important stabilizing effect on cancer (including chemotherapy-resistant disease) conferring prolonged clinical benefit and to have positive effects on the quality of life of patients with various types of cancer, without high-grade toxicity [48]. Moreover, low cost and oral administration (which reduces the need for hospitalization and enables patients to stay at home longer) are key characteristics of this schedule, offering important advantages in frail subgroups of patients (e.g., old patients) for whom new therapeutic options are greatly needed.

19.2 Metronomic Chemotherapy in Dogs

Based on the abovementioned studies, metronomic chemotherapy is increasingly being considered by veterinary oncologist as an anticancer therapeutic strategy. The first report of a metronomic chemotherapy protocol was presented as an abstract at the annual conference of the Veterinary Cancer Society a decade ago [49]. In that work, different types of tumors were treated with cyclophosphamide at 25 mg/m^2 in combination with piroxicam 0.3 mg/kg daily. An objective response was achieved in two dogs after 1 month of therapy.

The first full paper was not published for another 6 years [50]. It compared the outcome of nine dogs with splenic hemangiosarcoma treated with a traditional doxorubicin protocol dose-intense single- agent chemotherapy and nine dogs with the same disease treated with metronomic chemotherapy. The protocol consisted of piroxicam 0.3 mg kg in combination with cyclophosphamide or etoposide at 12.5–25 mg/m^2 to 50 mg/m^2 administered daily for 6 months. Surprisingly, the median survival time and, to an even larger extent, the disease-free interval proved better with the metronomic protocol than with the traditional one, resulting in 178 days as compared to 133, and 178 days versus 126, respectively [50].

However, this study provided important information on toxicity. Toxicity did not exceed grade 2 according to the VCOG [51] (Table 19.1) with the metronomic chemotherapy, while gastroenteric toxicity was observed at grades 3 and 4 with doxorubicin. However, hemorrhagic cystitis was reported in two patients treated with cyclophosphamide.

A year later Elmslie et al. [52] published a study of 85 dogs. Participating dogs had a soft tissue sarcoma that had been incompletely excised; one group was treated with metronomic chemotherapy (30), while the other group received no treatment (55). The protocol consisted of cyclophosphamide at 10 mg/m^2 combined with piroxicam 0.3 mg/kg daily or on alternate days. The treated group had a disease-free interval almost double that of the untreated group (410 versus 211 days). Toxicity was reported as mild, about 40 % of animals were reported as having grade 1 or 2 signs, and only one subject developed hemorrhagic cystitis (grade 4).

The next paper on metronomic treatment was published in early 2012. Fifteen dogs with various tumors with distant metastases were treated with metronomic chemotherapy as first-line treatment [38]. The protocol consisted of daily administration of cyclophosphamide, 25 mg/m^2, and celecoxib, 2 mg/kg. Six dogs obtained objective responses, including one complete remission. The average survival time was more than 3 months and toxicity almost absent. In this study, VEGF was measured before treatment and dogs who responded to the protocol showed a statistically significant lower blood concentration if compared to dogs who didn't respond.

In the same year, a study on 81 dogs with inoperable tumors, or tumors that had been incompletely removed or were chemoresistant with macroscopic evidence of distant metastases, was published [53]. These dogs were treated with lomustine at 2.84 mg/m^2 daily given orally for an average of 98 days. In almost half the cases, lomustine was used in conjunction with an anti-inflammatory drug; in 29 dogs an NSAID was used. Results obtained using this protocol were comparable to those

Table 19.1 Some criteria for adverse events following chemotherapy (VCOG-CTCAE)

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Anorexia	Coaxing or dietary change required to maintain appetite	Oral intake altered (<3 days) without significant weight loss; oral nutritional supplements indicated	Of 3–5 days duration; associated with significant weight loss or malnutrition; IV fluids, tube feeding, or TPN indicated	Life-threatening consequences; >5 days duration	Death
Diarrhea	Increase of >2 stools per day over baseline	Increase of 2–6 stools per day over baseline; parenteral (IV or SC) fluids indicated <24 h; not interfering with ADL	Increase of >6 stools per day over baseline; incontinence; IV fluids >24 h; hospitalization; interfering with ADL	Life threatening (e.g., hemodynamic collapse)	Death
Vomiting	<3 episodes in 24 h	3–5 episodes in 24 h; <3 episodes/day for >2 days but <5 days Parenteral (IV or SC) indicated <24 h	>5 episodes in 24 h; vomiting >4 days IV fluids or PPN/TPN indicated >24 h	Life threatening (e.g., hemodynamic collapse)	Death
Cystitis	Asymptomatic; microscopic hematuria/pyuria	Pollakuria with dysuria; macroscopic hematuria	Transfusion indicated; pain or antispasmodic medication; bladder irrigation indicated	Catastrophic bleeding; nonelective intervention indicated	Death
Urinary frequency	> in frequency or nocturia up to 2× normal	>2× normal but < hourly	≥ 1×/h	–	–
Packed cell volume (PCV)	Dog: 30 %–<LLN Cat: 25 %–<LLN	Dog: 25–<30 % Cat: 20–<25 %	Dog: 20–<25 % Cat: 15–<20 %	Dog: <20 % Cat: <15 %	–
Neutropenia	1,500/mL–<LLN	1,000–1,499/mL	500–999/mL	<500/mL	–

ADL activities of daily living (eating, sleeping, defecating, and urinating), *LLN* lower limit of normal

obtained by Marchetti et al. [38], with a response rate of 36 %. The nonprogression of the disease, observed in approximately 30 % of cases, had a duration of about 137 days. Even the toxicity evaluation aligns with the studies cited above with mild gastrointestinal symptoms occurring in about a quarter of patients. In about 21 % of cases, there was an increase in ALT, probably because of liver toxicity induced by lomustine. Finally, thrombocytopenia was found in about one-fifth of the cases; however, this was rarely higher than second-degree toxicity. This predominantly occurred in patients with end-stage cancer, probably due to a cumulative toxicity.

A prospective study was designed to observe the efficacy of metronomic dosing of cyclophosphamide to reduce the number of circulating Treg cells and tumor microvessel density (MVD, microvessel density) in dogs with soft tissue sarcoma (STS soft tissue sarcoma). Eleven dogs were treated daily with 12.5 mg/m^2 (5 cases) or 15 mg/m^2 (6 cases) of cyclophosphamide per os, while 21 healthy dogs were used as a control. The results showed significant efficacy in reducing the number of Treg lymphocytes circulating at both doses, while only the group treated with 15 mg/m^2 showed a reduction in MVD [14].

In a recent study, chlorambucil was administered at a dose of 4 mg/m^2 daily in dogs with spontaneous cancer [54]. Of 36 cases, 58 % recorded an objective response, with an overall mean survival time of 153 days. As in other trials, the observed toxicity did not exceed grade 2.

A very recent study has evaluated the effectiveness of low-dose cyclophosphamide with or without piroxicam in inhibiting tumor neovascularization and vascular normalization using a canine oral malignant melanoma-xenografted mouse model. Proliferation index, microvessel density, and VEGF levels are significantly reduced by treatment [55].

In a prospective study [56], 15 dogs with advanced malignancies were subjected to an experimental chemotherapy protocol that included a combination of metronomic cyclophosphamide (15 mg/m^2) and toceranib, a tyrosine kinase inhibitor recently approved for the treatment of canine mast cell tumors [57, 58]. The purpose of the study was not to observe the clinical response to treatment but rather to observe change in the number of circulating Treg cells and blood concentrations of interferon gamma (IFN-γ). The period of follow-up monitoring was short at 8 weeks; during this period 6 objective responses occurred, defined as stable disease and gastrointestinal and hematological toxicity no higher than grade 2.

19.3 Drug Candidates for Combinations with Metronomic Chemotherapy in Dogs

In human therapy in recent years, considerable interest has developed in drugs that could be easily used in combination with metronomic chemotherapy, such as nonsteroidal anti-inflammatory drugs (NSAIDs) [59] and tyrosine kinase inhibitors [60]. COX-2 is normally present in a limited number of tissues; it is mainly expressed in pathological states such as in inflammatory reactions and in tumors upon stimulation by inflammatory mediators such as interleukin-1 (IL-1), TNF-1, and

lipopolysaccharides [61]. In humans, the contribution of PG to tumorigenesis [62] has been also described. PGE2 has been shown to exert its action by binding to a specific class of receptors on the cell surface, called EP. Through this binding, it influences a series of intracellular events that lead to tumor development. These include induction of cell proliferation and increased cell survival via inhibition of mechanisms of apoptosis. PGs also promote angiogenesis through the production of growth factors such as VEGF and bFGF. Cumulatively, this leads to increased invasion and metastatic capacity and suppression of the immune response [62–65].

In dogs, several studies have shown increased expression of COX-2 in tumor tissues such as breast cancer [66–69], prostate cancer [70–73], transitional cell carcinoma [74–76], squamous cell carcinoma [71], and many other forms of tumor [71, 77–84]. NSAIDs have demonstrated antitumor efficacy in vitro [85–87] and they have been used in some clinical trials initially as a single agent [72, 88–91], revealing a response rate ranging from 17 to 33 %. This rate increased to 47–83 % if the objective was stable disease. These drugs were used in intensive chemotherapy regimens for oral squamous cell carcinomas and melanomas with a response rate of 25 % [77].

Another class of drugs subject to much interest in the veterinary field in the last decade is tyrosine kinase inhibitors (TKI). The tyrosine kinase receptors (TKR) are proteins that generally occur as monomers on the cell surface. They play a key role in the normal cellular signal transduction, regulating growth and cell differentiation. TKR interacts with adenosine triphosphate (ATP) by adding a phosphate group to its residues ("autophosphorylation") and onto other molecules to generate intracellular signals that influence proliferation and cell survival [92]. This process generally begins in response to external signals generated by growth factors or other stimuli that trigger the cascade of tyrosine phosphorylation.

The TKR are aberrant in many tumors in dogs and humans. The anomalies include overexpression, activating mutations, and autocrine activation through the co-expression of the receptor and the growth factor [93]. This causes continuous stimulation of cellular signals that induce altered proliferation and cell survival, even in the absence of adequate stimulation [58].

The most studied TKR is certainly TKI or CD-117 in the dog. It is often mutated in mast cell tumors [94] and gastrointestinal stromal tumors (GIST) [95] in the dog. Several other receptors such as VEGFR, PDGFR, and FGFR are included in the TKR family.

The TKI drugs are small molecules capable of binding selectively to and inhibiting the RTKs. These act by reversibly or irreversibly blocking binding sites for ATP on kinase enzymes [93, 96, 97]; in the absence of ATP binding, the kinase cannot work.

In veterinary medicine, few studies have been conducted investigating the efficacy of these anticancer drugs thus far. The use of imatinib [98, 99], masitinib [100], and toceranib [58, 94] for the treatment of mastocytomas has been tested in the dog, mainly investigating its inhibition of KIT. However, toceranib, which has demonstrated a wider range of target TKs, has also been used in the treatment of other tumors, such as lymphoma, breast cancer, carcinoma of the bladder transitional cell, soft tissue sarcoma, melanoma, osteosarcoma, hemangiosarcoma, squamous cell carcinoma of the tongue, multiple myeloma, bronchial carcinoma, sebaceous carcinoma, and anaplastic carcinoma [57].

Due to this property, but also others, toceranib is able to inhibit receptors in addition to KIT TK, such as VEGFR, PDGFR, and Flt-3. That has sparked interest in the use of TKIs as an antiangiogenic agent.

Other studies have also shown an immunomodulatory effect for this class of drugs. In particular, a recent study of 15 dogs affected by cancer, demonstrated toceranib's ability to reduce the number of circulating Treg lymphocytes, thus suggesting an additional antitumor action for this molecule [56]. This effect was already described in humans with sunitinib [101]. Mitchell et al. [56] tested toceranib as a single agent and subsequently it was combined with cyclophosphamide in a metronomic regime. No significant difference was observed in the absolute number of Treg lymphocytes before and after drug treatment. It was speculated that a synergistic effect in maintaining low levels of circulating Treg took place.

Another drug of current interest to the scientific community due to its antiangiogenic properties in canine tumors is thalidomide. This drug has an immunomodulatory and antiangiogenic effect [102]. Its antiangiogenic effect on canine tumors has been demonstrated using canine osteosarcoma cells transplanted into athymic nude mice [103].

To our knowledge, to date, the only clinical study on the efficacy of thalidomide in veterinary oncology has involved an unresectable case of head and neck squamous cell carcinoma in a cat [104].

Conclusions

Given the encouraging results from various trials [38, 52, 53], metronomic chemotherapy is offered as the treatment of choice for all pets with malignant tumors where owners are reluctant to embark on an aggressive therapy protocol, including surgery with a high American Society of Anesthesiologists (ASA) score. It is also indicated in all patients with organ failure such as hepatic or renal insufficiency, in which the toxicity of chemotherapy may be fatal. Metronomic therapy could also present an attractive option in patients with an aggressive nature that would require sedation for each parenteral administration.

In veterinary medicine, metronomic chemotherapy has several advantages compared to regime intense chemotherapy, especially in the context of veterinary oncology. There is minimal impact on the animal, cost is low, and administration is simple. Chemotherapy in low doses can be administered at home with minimal stress on the patient and minimal impact on the logistic organization of the owner. As cost of treatment is influential in choosing a therapeutic option, this type of chemotherapy, as compared with dose-intense protocols, has an unquestionable advantage. It has a low cost, approximately 1/10th of the cost of an intense scheme therapy.

Last, but not least, metronomic therapies have been shown to infrequently cause toxicity in veterinary patients [38, 50, 53, 54], except for an isolated case of hemorrhagic cystitis [52]. This contributes to the cost-effectiveness of such a protocol as it rarely requires the use of medications to treat the side effects and it results in much less time in hospital, both events that contribute to the higher overall costs of a dose-intense protocol.

In summary, metronomic chemotherapy achieves the main goal of veterinary oncology: good quality of life of the patient, with an affordable cost/benefit ratio.

It can be also offered as an alternative to dose-intense chemotherapy in cases where the owners are not able to manage any side effects or where simple precautions required for elimination of drugs cannot be complied with.

Finally, it is certainly proposed as being at the forefront of palliative care for patients at an advanced clinical stage, given that the objective in these cases is usually to stabilize disease with the least possible impact on the individual.

Unfortunately, the dose of drugs desirable for veterinary metronomic therapy has to be individually tailored. They can be prepared on demand by authorized pharmacies only. It is dangerous for anyone to split/partition tablets/capsules containing antineoplastic drugs and it is absolutely vital that veterinarians inform owners of the danger involved in use of these drugs at home. It is strongly recommended that owners are informed how to administer, store, and label the drug. It is also pivotal that they are aware of how to manage an animal that is excreting antineoplastic drug which continues for several days after the last administration.

Cancer in dogs shares many features with human cancer, including histological appearance, tumor genetics, molecular targets, biological behavior, and response to conventional therapies, so much so that the dog is considered a good model of human pathology [59, 105]. The initiation and tumor progression in both species is influenced by similar factors including age, nutrition, sex, reproductive status, and environmental exposure; for the latter in particular, the dog is considered a sentinel of environmental exposure because of its shorter life span [106–108]. Furthermore, most, if not all, of the cancer-associated genetic alterations that influence cancer progression in humans have been identified in canine cancer. Thus, the genome of the dog and human are similar enough to suggest that information learnt about one species can be extrapolated and applied to the other [109, 110]. Many of the chemotherapy protocols used in veterinary medicine are based upon protocols used in human patients and they have similar treatment outcomes. For this reason, dogs serve as unique animal models for some human tumors, because they adequately mimic many of the features that define cancer in humans, including long periods of latency, genomic instability, and an intact immune system [111].

Thus, the metronomic chemotherapy experience in dogs could reveal innovative and unexplored schedules for humans. Veterinary oncology cases treated with metronomic schedules represent the unique opportunity to ethically investigate novel drugs or combination treatments that may be highly translatable to the human community.

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