10 Metronomic Chemotherapy: Principles and Lessons Learned from Applications in the Treatment of Metastatic Prostate Cancer

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Abstract By frequent and protracted administration of conventional cytotoxic drugs without prolonged interruptions, the primary treatment target shifts from the tumor cell population to the tumor vasculature. This "metronomic" way of chemotherapy administration results in antivascular effects, the mechanistic basis of which remains to be fully elucidated. We outline the basic aspects of the metronomic concept, describe the results of clinical applications of such chemotherapy by focusing on studies in metastatic prostate cancer, and discuss certain shortcomings. Based on preclinical findings, we finally point to the possible ways to address these shortcomings in order to bring this novel and promising use of conventional anticancer agents to full fruition.

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10.1 Introduction

The concept of using antiangiogenic therapies as an anticancer strategy was formulated in 1971 by Folkman (1971) and clinically validated three decades later with the first successful application of an antiangiogenic agent, that is, the vascular endothelial growth factor (VEGF) targeting monoclonal antibody bevacizumab (Hurwitz et al. 2004). During this period, a sometimes tortuous path of discoveries led to an ever-increasing understanding of the complex process of tumor angiogenesis (Kerbel 2000). At present, the use of antiangiogenic agents is considered (part of) the standard of care for the treatment of colorectal, nonsmall cell lung, breast, kidney, and hepatocellular cancer, and involves the use of drugs such as bevacizumab and the antiangiogenic small molecule receptor tyrosine kinase inhibitors sunitinib and sorafenib (Kerbel 2006; Zhu 2008).

One interesting finding of the development of antiangiogenic therapies was that some targeted agents that were originally not developed as antiangiogenic drugs have been found to have "accidental" antiangiogenic properties (Kerbel et al. 2000). Furthermore, most conventional cytotoxic drugs can exert significant

R. Liersch et al. (eds.), *Angiogenesis Inhibition,* Recent Results in Cancer Research,
DOI: 10.1007/978-3-540-78281-0_10,

antiangiogenic effects (Miller et al. 2001). However, when chemotherapeutics are used in a conventional manner (i.e., bolus drug administration followed by a 3–4-week drug-free period to allow the host to recover from the adverse side effects), the vascular damage inflicted by the cytotoxic drug(s) is thought to be rapidly repaired during the recovery period, thus negating any significant overall antiangiogenic effects. Conversely, Browder et al. showed that by shortening the drug-free break period between individual chemotherapy administrations, the net antiangiogenic effects of conventional cytotoxic drugs can be largely augmented (Browder et al. 2000). In addition, we showed that this form of antiangiogenic chemotherapy, commonly referred to as metronomic chemotherapy (Hanahan et al. 2000), is more potent when combined with targeted antiangiogenic agents, especially drugs that interfere with the endothelial cell survival activity of VEGF (Klement et al. 2000). As summarized by Kerbel and Kamen (2004), metronomic chemotherapy protocols are generally characterized by:

- Frequent (dose-dense) and regular (metronomic) – often daily – chemotherapy administration without any prolonged interruptions.
- Absence of a dose-escalation up to the maximal tolerated dose (MTD).
- Absence of the need for hematopoietic growth factor support.
- Preference for oral, outpatient regimens by using drugs such as cyclophosphamide (CPA).
- Low incidence or absence of treatment-related side effects.
- Potential for delayed emergence of resistance.

Because of mostly inconsequential side effects, metronomic regimens can be coadministered with targeted therapies for prolonged periods of time. Furthermore, the use of inexpensive, off-patent drugs such as CPA results in reduced costs compared to many MTD chemotherapy regimens (Bocci et al. 2005).

The feasibility and clinical benefits of this novel use of conventional cytotoxic drugs have been shown in various Phase II trials involving diverse tumor types such as breast, prostate, and ovarian cancer as well as non-Hodgkin's lymphomas among others (Colleoni et al. 2002; Glode et al. 2003; Burstein et al. 2005; Bottini et al. 2006; Buckstein et al. 2006; Colleoni et al. 2006; Young et al. 2006; Lord et al. 2007; Garcia et al. 2008). These findings remain to be confirmed in Phase III trials. Furthermore, important questions remain to be addressed such as the optimal dose and most effective dosing interval, improved monitoring of the antiangiogenic effects, the choice of cytotoxic drugs used for a given tumor type, and the most efficacious way to integrate metronomic chemotherapy into standard therapy protocols.

We provide an overview of the molecular mechanisms behind the antivascular effects of metronomic chemotherapy, and discuss clinical results as well as some shortcomings of the metronomic concept by focusing on published applications for the treatment of metastatic castration-resistant prostate cancer (CRPC). Finally, we discuss the potential future role of metronomic as compared to conventional MTD chemotherapy.

10.2 Mechanisms of Action of Metronomic Chemotherapy

Experimental evidence that chemotherapy administered in a condensed schedule slows down the repair of the drug-induced damage to the tumor vasculature was first reported in 2000. Browder et al. showed that CPA administered every 6 days produced more sustained antiangiogenic effects compared to conventional every 3-week MTD CPA administration (Browder et al. 2000). Intriguingly, CPA was even effective in tumors that had been made resistant

in vivo to a conventional CPA regimen, further suggesting that mechanisms other than direct antitumor effects are the basis of the antitumor effects seen with metronomic protocols. In addition, when mice bearing large, established human neuroblastoma xenografts were treated by Klement et al. with twice weekly metronomic administrations of vinblastine combined with DC101, a monoclonal antibody blocking the murine vascular endothelial cell growth factor receptor 2 (VEGFR2), this combination therapy resulted in a significant therapeutic benefit (Klement et al. 2000). Tumors completely regressed over time and did not relapse during a 7-month period of uninterrupted therapy. Both studies suggest that metronomic regimens act largely by inhibiting tumor angiogenesis.

10.2.1 Preferential Antiproliferative Effects of Metronomic Chemotherapy Toward Endothelial Cells

In vitro studies indicated that a 6-day continuous exposure of human micro- and macrovascular endothelial cells to low concentrations of chemotherapy drugs such as paclitaxel or the CPA precursor 4-hydroperoxy-CPA resulted in preferential endothelial cell growth inhibition compared to other cell types, for example, human fibroblast and breast cancer cells (Bocci et al. 2002). These results provided further evidence that metronomic regimens using various chemotherapy drugs may have a highly selective effect against rapidly dividing vascular endothelial cells.

Subsequently, we reported that protracted in vitro exposure of endothelial cells to low concentrations of several cytotoxic agents causes a marked induction in the expression of thrombospondin-1 (TSP-1) at the mRNA and protein level (Bocci et al. 2003). TSP-1 is a potent endogenous inhibitor of angiogenesis, which acts primarily by binding to endothelial cells expressing the CD36 receptor, resulting in the induction of endothelial cell death (Volpert et al. 2002; Yap et al. 2005). TSP-1 also exerts indirect antiangiogenic effects by binding and sequestering VEGF (Gupta et al. 1999). With regard to metronomic chemotherapy in vivo, induction of circulating TSP-1 plasma levels was observed in mice bearing human xenografts that were treated with metronomic CPA (Bocci et al. 2003). Further evidence for the role of TSP-1 was obtained by administering metronomic CPA to TSP-1 knockout mice bearing Lewis lung carcinoma. Compared to wild-type mice, the metronomic regimen lost its antitumor activity in the knockout mice. However, when CPA was administered at the MTD, retention of the antitumor effects in both wild-type and TSP-1 deficient mice was observed. Similar results were obtained by another group when CPA was administered on a weekly basis to TSP-1 knock-out mice bearing B16 mouse melanoma, and others (Hamano et al. 2004; Damber et al. 2006; Ma and Waxman 2007; Ma and Waxman 2008). Interestingly, the antitumor effects of metronomic regimens were retained in mice that were unable to produce either endostatin or tumstatin, both of which are other endogenous inhibitors of angiogenesis (Hamano et al. 2004). Taken together, these results suggested that TSP-1 is a mediator of the antiangiogenic effects of at least some metronomic regimens and confers endothelial cell specificity.

10.2.2 Circulating Bone Marrow-Derived Endothelial Precursor Cells as Targets of Metronomic Chemotherapy

In addition to *angiogenesis* mediated by local sprouting of rapidly dividing endothelial cells from pre-existing capillaries, the tumor vasculature also depends on *vasculogenesis* mediated by circulating endothelial precursor cells (CEPs) originating from the bone marrow. Following

mobilization, CEPs enter the blood circulation and subsequently home to sites of active angiogenesis, where they differentiate and incorporate into the lumen of growing blood vessels and proliferate (Bertolini et al. 2006). A number of preclinical studies suggest that certain tumors are highly dependent on this vasculogenic support, namely lymphomas, Ewing's sarcomas, and inflammatory carcinomas of the breast (Bertolini et al. 2003; de Bont et al. 2001; Bolontrade et al. 2002; Shirakawa et al. 2002). For example, using NOD SCID mice bearing Namalwa or Granta 519 human lymphomas, Bertolini et al. demonstrated that shortly after the administration of an intensive MTD course of CPA, levels of CEPs were substantially reduced for the first few days, followed by a marked rebound during the drug-free break period. This rebound and its timing are reminiscent of the process of hematopoietic recovery after myelosuppressive therapy (Bertolini et al. 2003). In contrast, when CPA was administered in a metronomic regimen, that is, either injected i.p. every 6 days (Browder et al. 2000) or continuously via drinking water (Man et al. 2002), levels of CEPs gradually declined and remained suppressed during the entire treatment period. The degree of mobilization of CEPs and their viability during treatment with MTD versus metronomic CPA is depicted in Fig. 10.1.

Interestingly, we reported a similar or even more important CEP rebound after treatment with vascular disrupting agents (VDAs) (Shaked et al. 2006). As opposed to antiangiogenic agents, this class of drugs causes an acute occlusion of tumor blood vessels, which subsequently results in tumor cell death. However, some remaining viable tumor tissue is usually observed at the rim, from which tumor growth rapidly resumes. We have shown that CEPs can be mobilized from the bone marrow in a matter of hours of treatment with a VDA, and subsequently home to the viable tumor rim. CEPs then incorporate into the tumor blood vessels and promote angiogenesis, which results in rapid tumor repopulation. Taken together, the surge of CEPs following MTD chemotherapy or treatment with a VDA could contribute to the vascular repair process referred to by Browder et al. (2000). Conversely, the metronomic administration of cytotoxic drugs may inhibit the CEP rebound phenomenon and promote the antiangiogenic effects of such chemotherapy. Suppression of the CEP surge is one of the mechanisms that might account for the beneficial effects seen when metronomic or other antiangiogenic therapies are combined with MTD chemotherapy (Kerbel 2006).

10.2.2.1 Benefit of Combined Bolus and Metronomic Chemotherapy Administration

One aspect of the aforementioned study by Bertolini et al. (2003) was whether the combination of metronomic with intermittent bolus dose chemotherapy administration could be effective as a long-term antitumor treatment strategy. We hypothesized that the metronomic regimen might inhibit the CEP rebound following bolus chemotherapy administration. Using three different tumor models, that is, human prostate cancer xenografts (PC-3), Friend virus-induced murine erythroleukemia, and murine breast cancer (EMT-6), we demonstrated that the combination of bolus dose CPA (in our case one-third of the conventional MTD administered every 3 or 6 weeks) plus metronomic CPA was more effective than MTD or metronomic monotherapy (Shaked et al. 2005b). Importantly, the levels of CEPs remained suppressed despite bolus CPA administration. In another study, Pietras and Hanahan reported similar findings. Briefly, a "chemo-switch" regimen (defined by upfront MTD CPA, followed by metronomic CPA combined with targeted antiangiogenic agents) produced significant antitumor responses and survival benefits in a mouse pancreatic cancer model (Pietras and Hanahan 2005).

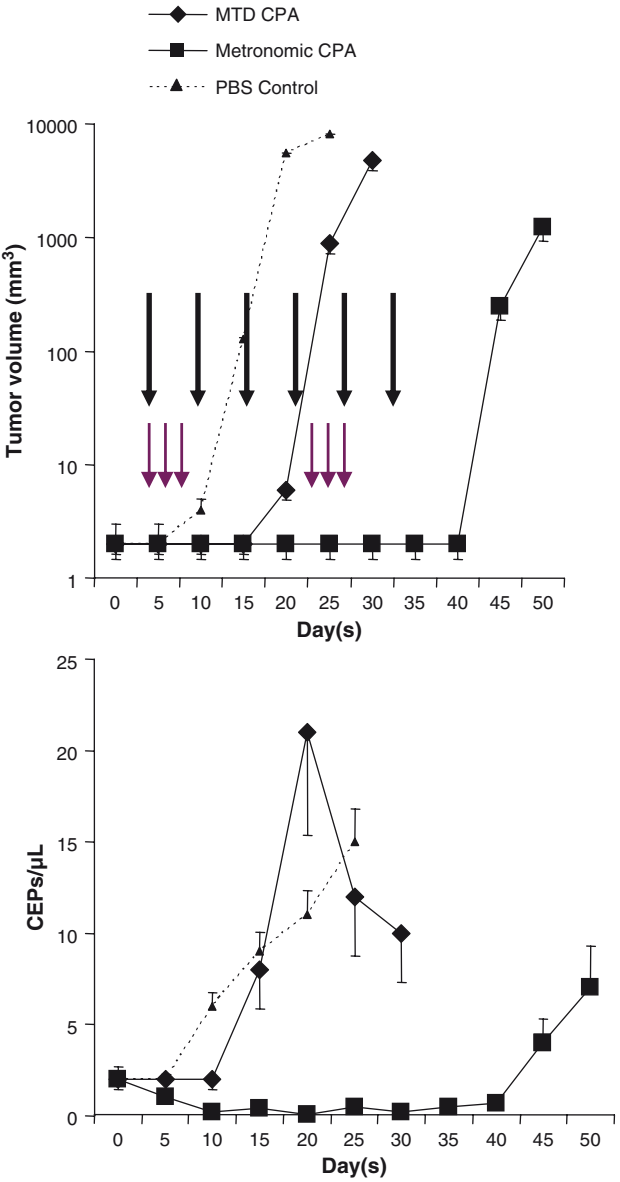

Fig. 10.1 The effect of metronomic and MTD chemotherapy regimens on CEP levels. Human Namalwa lymphoma bearing NOD/SCID mice were treated with CPA administered either at the MTD for this mouse strain, i.e., 75 mg/kg i.p. injection every other day for three doses per cycle (*purple arrows*), or as a metronomic regimen, i.e., 170 mg/kg i.p. injection every 6 days (*black arrows*). Tumor volumes (*upper graph*) and levels of CEPs detected in peripheral blood (*lower graph*) were monitored regularly (adopted with minor modifications from Bertolini et al. 2003, with permission from the publisher)

10.2.2.2 CEPs and Optimal Biological Dose of Antiangiogenic Agents

Evidence for antivasculogenic effects of metronomic chemotherapy was also reported in our study that sought to determine whether CEPs can serve as a pharmacodynamic biomarker to determine the optimal biological (=antiangiogenic) dose of antiangiogenic drugs or treatment strategies. The fact that CEPs gradually declined after treatment with metronomic CPA

(Bertolini et al. 2003) led us to investigate whether the levels of such circulating cells may reflect the level of antivascular activity in mice. A previous study had demonstrated that various mouse strains exhibit different levels of angiogenic responsiveness as measured by the corneal micropocket assay (Rohan et al. 2000). The angiogenic stimulus, that is, basic fibroblast growth factor (bFGF), was implanted into the corneas of different mouse strains and vessel growth/sprouting was evaluated. Strains such as C57Bl/6 exhibited a low number of sprouting vessels (indicating a low level of angiogenic responsiveness), whereas others strains, for example, BALB/c or 129, showed a very strong angiogenic response. In our studies, we evaluated the baseline CEP levels in different mouse strains and found a striking correlation between the number of such cells in peripheral blood and the angiogenic responsiveness previously determined for the same strains with the corneal micropocket assay (Shaked et al. 2005a). These results suggest that CEPs might be used as a biomarker to determine the level of angiogenic activity in mice. Subsequently, CEP levels were measured 1 week after treatment with antiangiogenic drugs such as DC101 or ABT-510, a TSP-1 mimetic peptide (Shaked et al. 2005a). In both cases, we found that the drug doses producing maximum antitumor activity also caused the greatest decline in viable CEPs. Similar results were obtained with metronomic regimens using various chemotherapy drugs, for example, CPA, vinblastine, vinorelbine, cisplatinum, ABI-007 (Abraxane®, a cremophor-free nanoparticle paclitaxel preparation), and UFT (Uftoral®, tegafur-uracil), administered to mice bearing various human tumor xenografts (Shaked et al. 2005c; Munoz et al. 2006; Ng et al. 2006). In fact, after a single week of treatment we found a striking correlation between the metronomic drug dose resulting in maximal antitumor activity without overt toxicity and the greatest decline in CEP levels in peripheral blood (Shaked et al. 2005c).

10.2.3 Mechanisms of Action Summarized

Figure 10.2 demonstrates some of the possible mechanisms of action of metronomic chemotherapy regimens. Thus far, such regimens have mostly been investigated with respect to their antivascular effects involving the inhibition of both the locally dividing activated endothelial cells and the systemic vasculogenic process mediated by CEPs. However, much needs to be learned about the effects of metronomic therapy on other bone marrow cell types that might promote angiogenesis or tumor growth via different mechanisms, possible direct effects of metronomic regimens on tumor cells, potential immunomodulatory activities of drugs like CPA, in particular when used in a protracted manner (Ghiringhelli et al. 2007), and possible adverse side effects (Fig. 10.3).

10.3 Metronomic Chemotherapy for the Treatment of Metastatic Castration-Resistant Prostate Cancer

Protracted cytotoxic drug administration was studied as early as in the 1970s (Vogelzang 1984). However, such chemotherapy regimens often included regular treatment-free breaks and the dosing was oriented toward maximizing the cytototoxic effects. Since the first preclinical descriptions in 2000 (Browder et al. 2000; Klement et al. 2000), the results of more than 50 clinical trials embracing the metronomic concept have been published. Breast and prostate cancer are among the best studied tumor types in this respect (Colleoni et al. 2002; Glode et al. 2003; Burstein et al. 2005; Bottini et al. 2006; Colleoni et al. 2006; Lord et al. 2007).

CRPC is particularly well suited for a metronomic chemotherapy type of treatment strategy. The role of angiogenesis in prostate cancer in

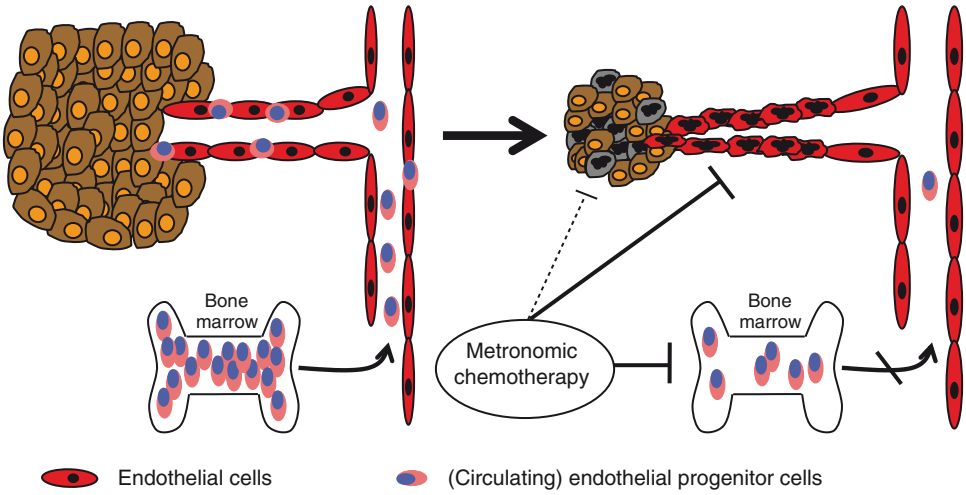

Fig. 10.2 Antivascular effects of metronomic chemotherapy. Metronomic chemotherapy affects dividing, activated tumor endothelial cells and inhibits the mobilization and/or the viability of bone marrow derived CEPs, which can contribute to tumor neoangiogenesis. Furthermore, drugs like CPA used in a protracted, low-dose manner might also exert immunomodulatory effects. Direct anti-tumor effects seem not to play a major role in most instances when chemotherapy is given in a metronomic manner (modified from Shaked et al. 2005c, with permission from the publisher)

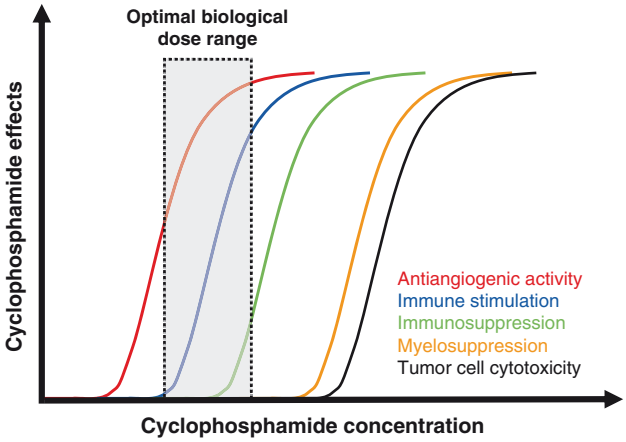

Fig. 10.3 Hypothetical dose-response curves of various metronomic CPA effects. When given at the optimal biological dose, metronomic CPA results in antiangiogenic and possibly also immunostimulatory effects. Higher CPA doses increase the risk of immuno/myelosuppression, likely without added benefit as far as antiangiogenic effects are concerned

general, and in the castration-resistant stage in particular, is well documented (Nicholson and Theodorescu 2004). Furthermore, CRPC is mainly a disease of the elderly where treatment-related adverse effects may limit the use of overly toxic approaches (Pienta and Smith 2005). Historically, the focus on quality of life aspects rather than improving overall survival has been a necessity in the treatment of CRPC in the absence of therapies impacting on the latter (Tannock et al. 1996). The current standard MTD chemotherapy approach, that is, 3-weekly administration of docetaxel-based chemotherapy (Petrylak et al. 2004; Tannock et al. 2004),

results in prolonged overall survival, improved pain control, and better quality of life. However, the impact on survival is only a modest 2–3 month increase compared to the former standard therapy of mitoxantrone/prednisone (Tannock et al. 1996). Thus, there is a clear need for novel strategies in patients that are not considered suitable for docetaxel chemotherapy or those that develop severe docetaxel-related side effects. In addition, there is an unmet need for new approaches in the maintenance setting following maximal response to docetaxel (Lin et al. 2007). Metronomic and other antiangiogenic therapies might meet these needs and are also an interesting option in early CRPC, where the possible benefits of conventional cytotoxic therapy do not outweigh the risk of adverse side-effects and their potential impact on the quality of life.

10.3.1 From Bench to Bedside

Given the lack of feasible metastatic prostate cancer models, the benefit of using metronomic chemotherapy in advanced metastatic disease has thus far been studied preclinically in spontaneous metastatic breast cancer and melanoma models (Munoz et al. 2006; Cruz-Munoz et al. 2008). An unexpected lesson from such studies is that the primary tumor response is not necessarily indicative of the effects of metronomic treatment strategies against metastatic disease. Briefly, breast cancer xenografts were allowed to grow in mice as primary, orthotopically implanted tumors, or to develop (following surgical removal of primary tumors) into visceral metastatic disease. Both primary tumors and metastases were then treated with metronomic CPA and UFT, administered as monotherapies or in combination. The results showed that the combination of CPA and UFT did not improve primary tumor response compared to CPA alone. However, the same combination was highly efficacious against metastatic disease involving multiple organs. Similar results were obtained with vinblastine and CPA in a metastatic melanoma model (Cruz-Munoz, Man and Kerbel, unpublished observations). Thus, had the analysis been carried out only on a primary tumor model, it would likely have suggested the erroneous interpretation that UFT (or vinblastine) was ineffective in improving the anticancer benefits of metronomic CPA monotherapy. These results highlight the importance of consideration that is needed to identify new metronomic combinations, and the importance of assessing them in appropriate disease models including metastatic cancer. Restricting studies to primary tumors may result in novel metronomic regimens being erroneously discarded as ineffective. For the same reason, it is therefore important that better metastatic prostate cancer models be developed to carry out similar studies in prostate cancer.

In attempting to develop improved metronomic therapies and compare them to conventional chemotherapy administration, another factor might be relatively overlooked, that is, the lack of significant observable host toxicity resulting from metronomic regimens, particularly when compared to conventional MTD chemotherapy. The limited preclinical studies of metronomic regimens against metastatic disease have thus far confirmed this finding. The toxicity aspect has hitherto been little appreciated because host toxicity is seldom a limiting factor in the design and execution of preclinical studies involving primary tumor xenografts. If the impact of treatment-related toxicity is not considered, the benefit of metronomic treatment on survival in preclinical metastatic disease might be underappreciated when compared to standard MTD regimens. Although this assumption remains yet to be formally tested in metastatic models, there already is confirmatory evidence from long-term therapy studies involving metronomic vs. MTD dosing in primary tumor models by du Manoir et al. and Shaked et al. (2005b; du

Manoir et al. 2006). Thus du Manoir et al. treated a human breast cancer model with trastuzumab (Herceptin®) plus CPA, where the alkylating agent was administered either metronomically or in a MTD fashion. Shaked et al. described a detailed comparison of MTD CPA with a metronomic CPA regimen that included interspersed bolus administrations of CPA at one third of the MTD in order to minimize toxicity. Both studies showed tumor responses that are schematically shown in Figs.10.4a, b. When tumor volume measurements were analyzed, there was indication that MTD CPA therapy was more effective against primary tumor growth than the metronomic-based regimen, particularly over a short treatment period (e.g., less than 50 days). This is important since currently most preclinical studies are completed within a relatively short time frame in which it is not unusual for the treatment to involve only one or two cycles of MTD therapy. However, over a longer treatment period, the mice on the MTD therapy had to be sacrificed. This was not because of complications arising from tumor growth, but because of overt toxicity, exemplified by weight loss (Fig. 10.4c). When the toxicity and tumor response were jointly considered in a Kaplan–Meier analysis, as done by du Manoir et al. (du Manoir et al. 2006), MTD CPA therapy did not show as significant an advantage over the metronomic-based regimen. Indeed, the fact that the mice on the MTD-based regimen died after three cycles of MTD actually made the metronomic regimen look better in the Kaplan–Meier plot comparison (Fig. 10.4d). On the other hand, in the study by Shaked et al. it was noted that mice on the MTD CPA regimen bearing human prostate cancer xenografts died after nine treatment cycles

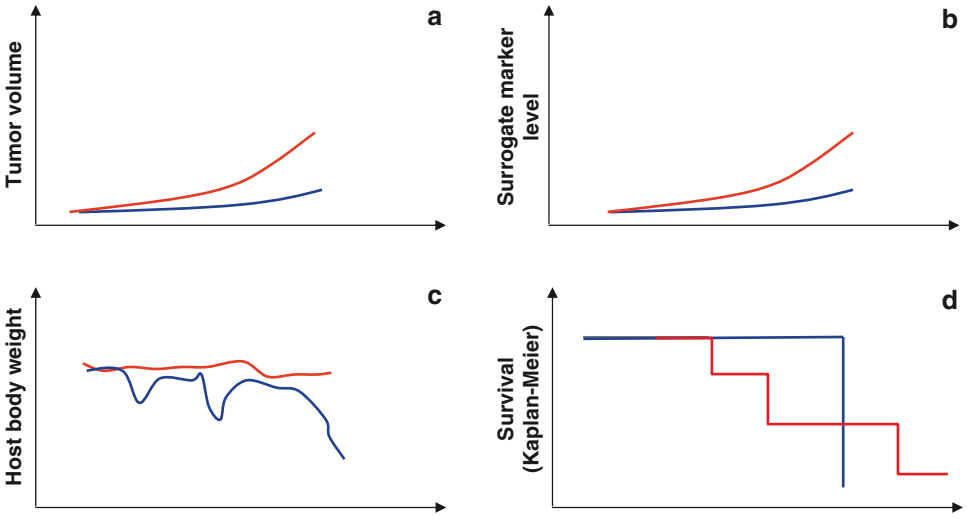

Fig. 10.4 Potential pitfalls in the design and interpretation of preclinical studies in mice using metronomic regimens. A number of studies have shown that in some cases, particularly over short periods of time, MTD (*blue lines*) can be more effective than metronomic (*red lines*) chemotherapy. This is something that would be assessed by (caliper based) tumor measurements (**a**), or surrogate marker analysis (**b**). However, such studies risk failing assessment of the relative impact of host toxicity which may only appear after a number of cycles of MTD administration, e.g., exemplified by progressive weight loss (**c**). This can be appreciated in a Kaplan–Meier plot (**d**), in which both toxicity and tumor growth are taken into account

due to toxicity (Shaked et al. 2005b). This is in sharp contrast to the metronomic CPA monotherapy regimen, which did not give rise to toxicity in the same mouse strain, even after several months of drug administration (Emmenegger et al. 2004; Emmenegger et al. 2006). Future preclinical metastasis studies reporting tumor size parameters and Kaplan–Meier plots will better define the contrast between MTD and metronomic dosing.

In clinical trials, parallel considerations may arise if a metronomic regimen proves equally effective to an MTD regimen in terms of survival, yet without the degree of toxicity that is often associated with MTD dosing (Rivera et al. 2008). To date, a number of clinical trials have reported the low incidence of high-grade toxic side effects with metronomic regimens (Colleoni et al. 2002; Bottini et al. 2006; Colleoni et al. 2006; Lord et al. 2007). Thus, one interesting possibility which would conceivably emerge is that metronomic chemotherapy protocols may be established as a valid alternative to MTD regimens, not because they are superior in prolonging survival, but because of an improved overall therapeutic benefit.

10.3.2 Key Findings of Metronomic Trials in Castration-Resistant Prostate Cancer and Emerging Questions

Table 10.1 summarizes the key findings of published metronomic CRPC trials (Nishimura et al. 2001; Glode et al. 2003; Nicolini et al. 2004; Di Lorenzo et al. 2007; Lord et al. 2007). With the exception of the study by Lord et al. (2007), many of the study subjects enrolled onto these trials had been previously exposed to conventional cytotoxic therapy. One of the drugs administered in all these studies is CPA, an agent that has been commonly used in the past for the treatment of CRPC using various nonmetronomic intravenous and oral regimens (Mike et al. 2006; Winquist et al. 2006). The applied CPA dose varied from 50 mg/day – the dose most commonly used in metronomic trials involving CPA (Kerbel and Kamen 2004; Gille et al. 2005) – to alternating 100/150 mg/day (Nicolini et al. 2004). Although PSA responses of >50% were typically rare, clinical benefit in the form of either minor PSA responses <50% or PSA stabilization were commonly seen and maintained for several months.

Toxicity has not been a major issue in metronomic trials for CRPC. In fact, grade 3–4 side effects are rare, the only exception being G3 lymphopenias seen in one-third of the patients in the study by Lord et al. (2007). The rather high daily CPA dose of 50 mg/m^2 used by these authors (representing ~100 mg/day for most patients) might be an explanation for this unexpected high rate of lymphopenia. However, it is reassuring that the lymphopenias described did not result in opportunistic infections. Although lymphopenias have been described as a consequence of metronomic CPA therapy in mice at the optimal biological dose (Emmenegger et al. 2004), the commonly used CPA dose in metronomic clinical trials of 50 mg/day seems to be devoid of this side-effect despite clear evidence of clinical activity (Colleoni et al. 2002; Glode et al. 2003; Burstein et al. 2005; Bottini et al. 2006; Buckstein et al. 2006; Colleoni et al. 2006; Garcia et al. 2008).

In the study by Lord et al., 22 patients received fewer than 8 weeks of CPA therapy. Most of them had to be removed from the study because of rapid disease progression (Lord et al. 2007). This is a reminder that this type of therapy needs to be used with caution in patients with rapidly progressive disease. An alternative might be to consider an initial intravenous bolus dose of CPA (or another cytotoxic agent) before commencing metronomic scheduling (Fontana et al. 2007).

In summary, metronomic studies in CRPC show reasonable clinical activity combined with a very appealing toxicity profile, findings that need to be confirmed in Phase III trials. Similarly,

Table 10.1 Metronomic Chemotherapy Trials in CRPC

Reference	Lord et al. 2007	Glode et al. 2003	Nishimura et al. 2001	Di Lorenzo et al. 2007	Nicolini et al. 2004
CRPC stage	Early	*Early*-advanced	*Early*-advanced	Advanced	Early-*advanced*
N=(evaluable)	80(58)	34(32)	21(21)	16(16)	8(8)
Treatment	CPA 50 mg/m^2 o.d.	CPA 50 mg o.d. Dexamethasone 1 mg o.d.	CPA 50 mg b.i.d UFT 200 mg b.i.d. EMP 280 mg b.i.d	CPA 50 mg o.d. THD 100 or 200 mg o.d.	CPA alternating 100 or 150 mg o.d. Mesna 400 mg p.o. 3/4 weeks
PSA response (%)	3	69	57	15	25
PSA stabilization (%)	N/A	6	24	8	37.5
Clinical benefit (%)	45	75	81	23	62.5
Grade 3–4 toxicities	Lymphopenia G3 32.8% Neutropenia G3 1.7% Anemia G3 1.7%	N/A	Leucopenia G3 14% Hemorrhagic cystitis G3 3.5% G3.5%	Neutropenia 20% Anemia 10%	N/A
Median response duration (range)	7.5 months (3–18)	8 months (95% CI: 4–10)	7 months (2–15)	Median survival 15 weeks (9–19)	9 months (8–31)
Comments	22 patients did not finish at least two treatment cycles (8 weeks) and were not included in the analysis	Retrospective analysis; treatment interruption in four patients (mainly for hematological toxicity); CPA withdrawn in 1 patient (gastro-intestinal symptoms)		All patients post two lines of cytotoxic therapy; only combined G3/G4 toxicity data presented	Best PSA response 3, 5, 7, 8, and 31 months after treatment initiation

CRPC castration-resistant prostate cancer; *N* number of patients; *CPA* cyclophosphamide; *UFT* tegafur-uracil; *EMP* estramustine phosphate; *THD* thalidomide; *N/A* not available; *clinical benefit*=complete response+partial response+stable disease

positive observations have been reported with other tumor types (Bottini et al. 2006; Buckstein et al. 2006; Colleoni et al. 2006; Garcia et al. 2008). However, these studies also raise many questions such as: What is the best choice of drug(s) used in such regimens? What is the optimal biological dose of a given drug? What are the most efficacious drugs or drug combinations to be used in metronomic protocols? How can metronomic regimens be integrated into current standards of practice?

10.3.2.1 Choice of Cytotoxic Drugs Used in Metronomic Regimens

CPA is an obvious choice for metronomic use in CRPC in that (a) the beneficial effects of metronomic CPA are well documented (pre)clinically (Man et al. 2002; Emmenegger et al. 2006), (b) the potential (long-term) side effects of CPA are well known from immunosuppressive protocols using CPA, albeit at higher daily doses than typically applied in metronomic regimens (Hoffman et al. 1992; Haubitz et al. 1998), and (c) CPA is available in an oral form. Furthermore, CPA has been used in the past for the treatment of CRPC, either orally or administered intravenously, albeit applying more conventional dosing schedules (Mike et al. 2006; Winquist et al. 2006). Interestingly, cytotoxic drugs successfully used in a conventional manner for a certain tumor type often also show clinical activity in metronomic protocols for the same tumor type. It remains to be seen whether this reflects certain tumor-related characteristics or whether it is rather a bias dictated by the experience of the prescribing oncologists. In fact, it is currently not known whether certain agents are more active in certain tumor types when used metronomically. Similarly, further study is needed to define whether the sequential use of cytotoxic drugs in metronomic regimens might be able to delay or overcome resistance (Kieran et al. 2005).

There is limited evidence that cytotoxics are not interchangeable when used as antiangiogenics. In fact, while inhibition of proliferation of endothelial cells seems to be likely a universal consequence of the metronomic use of chemotherapeutic agents (Bocci et al. 2002; Wang et al. 2003), other biological effects might be more drug-specific, such as the (a) TSP-1 induction by CPA and various microtubule inhibitors (Bocci et al. 2003; Hamano et al. 2004), (b) anti-Hif-1α activities of topoisomerase I inhibitors and microtubule inhibitors (Rapisarda et al. 2004; Escuin et al. 2005), and (c) the induction of CD95 on endothelial cells by agents such as doxorubicin and CPA (Quesada et al. 2005; Yap et al. 2005). Furthermore, pharmacokinetic characteristics might make certain chemotherapeutics drugs more or less suitable for metronomic use (Hahnfeldt et al. 2003).

Besides the question of which drug to use, it is also important to define patient and/or tumor characteristics that predict prolonged benefit from metronomic therapies. As an example, Orlando et al. have described advanced breast cancer patients receiving metronomic CPA/methotrexate with a median time to progression of almost 2 years (Orlando et al. 2006). In this study, patients achieving remissions or stable disease for 12 months or more comprised 15.7% of the entire study population.

10.3.2.2 Optimal Biological Dose

Preclinically, metronomic dosing often implies the frequent administration of ~1/3rd to 1/10th of the MTD of a given cytotoxic drug (Kerbel and Kamen 2004; Gille et al. 2005; Lam et al. 2006). More recently, a less empirical way to characterize the optimal biological dose was determined preclinically, that is, the dose with maximal CEP suppression in the absence of significant toxicity such as myelosuppression and body weight loss, as described earlier

(Shaked et al. 2005c). Unfortunately, the use of CEP levels for individual dosing is hampered in humans because of the lower number of such cells compared to mice (Bertolini et al. 2007).

Takahashi proposed the concept of the *individualized maximum repeatable dose* (Takahashi et al. 2005). Briefly, the weekly dose of gemcitabine was titrated to a dose resulting in stable Grade 1 toxicity despite prolonged gemcitabine use. As appealing as such an approach might be, it would likely be restricted to situations of metronomic monotherapy. As an alternative, the assessment of pharmacokinetic and/or pharmacodynamic parameters might become a way to tailor individual dosing in the future (Kamen et al. 2006; Emmenegger et al. 2007). However, despite major efforts there continues to be a lack of validated pharmacodynamic surrogate markers for antiangiogenic activity (Jubb et al. 2006).

Even when more sophisticated metronomic dosing might become feasible in the future, practical aspects need to be considered as well. As an example, in the metronomic clinical trial by Colleoni et al. in metastatic breast cancer, a daily CPA dose of 50 and 2.5 mg of methotrexate b.i.d. on Day 1 and 2 of every week were administered (Colleoni et al. 2002). The choice of these doses was driven by practical considerations such as available tablet size, and was assumed to facilitate a high level of patient compliance.

10.3.2.3 Combination Therapies

High levels of proangiogenic cytokines can confer endothelial cell resistance to the effects of cytotoxic drugs (Tran et al. 2002). Therefore, by combination with targeted antiangiogenic agents such as inhibitors of the VEGF pathway, the effects of metronomic chemotherapy can be augmented and vice versa (Klement et al. 2000; Burstein et al. 2005; Pietras and Hanahan 2005). In contrast to combinations involving MTD chemotherapy, which are generally limited to 6–10 continuous cycles, protocols involving targeted antiangiogenics combined with metronomic chemotherapy might be used for prolonged periods of time, given the excellent safety profile of such regimens (Bottini et al. 2006; Buckstein et al. 2006; Colleoni et al. 2006; Garcia et al. 2008). However, much needs to be learned about what type of drugs should be combined. For example, the combination of metronomic chemotherapy (CPA/methotrexate and CPA/vinblastine) with thalidomide or minocycline, respectively, two agents known to inhibit angiogenesis, seem not to be superior to metronomic chemotherapy alone (Colleoni et al. 2006; Young et al. 2006). On the other hand, the combination of bevacizumab with metronomic CPA has yielded very promising results in breast and ovarian cancer (Burstein et al. 2005; Garcia et al. 2008). Indeed, in the randomized Phase II trial of advanced breast cancer by Burstein et al. the bevacizumab plus metronomic CPA/methothrexate arm was superior compared to CPA/methotrexate therapy alone in terms of response rate and median time to progression (Burstein et al. 2005).

Besides doublet metronomic chemotherapy involving CPA and methotrexate, combinations of CPA and fluorinated pyrimidines are also showing promising clinical activity. The combination of metronomic CPA and UFT was clearly superior to monotherapy with either CPA or UFT in a preclinical model of advanced metastatic breast cancer (Munoz et al. 2006). A similar metronomic doublet of CPA and capecitabine combined with bevacizumab has been successfully applied for the treatment of advanced breast cancer, and seems to confirm the preclincial findings of Munoz et al. (2006). As far as the treatment of CRPC is concerned, Nishimura et al. successfully combined CPA with UFT and estramustine in a nonrandomized Phase II trial (Table 10.1) (Nishimura et al. 2001).

10.3.3 Integration of Metronomic Chemotherapy into Current Standards of Practice for Prostate Cancer

Metronomic chemotherapy has been generally studied in situations of advanced disease stages, with metastatic CRPC being a typical example (Kerbel and Kamen 2004; Gille et al. 2005). Such applications will likely continue to dominate in the near future. However, the results of a few studies suggest other indications worthy to be pursued.

For instance, metronomic chemotherapy might be considered as an adjunct to docetaxel chemotherapy, similar to other clinical trials which are comparing docetaxel monotherapy with docetaxel plus various antiangiogenics as first-line therapy in CRPC (Ryan et al. 2006). In fact, concomitant conventional and metronomic chemotherapy administration has shown to be beneficial preclinically (Shaked et al. 2005b) and clinically (Ellis et al. 2002; Casanova et al. 2004). As far as clinical results are concerned, the pilot study by Casanova et al. demonstrated the feasibility and activity of MTD vinorelbine and daily oral CPA in children with refractory or recurrent sarcomas. Furthermore, Ellis et al. described the use of continuous CPA combined with dose-dense doxorubicin in the adjuvant therapy of node-positive breast cancer patients, a promising regimen that is being further pursued in a Phase III trial.

An alternative to concomitant administration is the sequential use of MTD and metronomic chemotherapy, as preclinically described by Pietras and Hanahan (2005). Indeed, maintenance strategies following initial tumor debulking are actively studied in CRPC (Lin et al. 2007). Metronomic chemotherapy is an interesting treatment option in this respect, besides the use of various targeted agents.

Finally, beneficial effects of metronomic temozolomide combined with radiation therapy have been described for pediatric brain tumors (Sterba et al. 2002). Similarly, metronomic therapy might find a place in the CRPC setting when given concomitantly with radiation therapy.

Earlier stages of prostate cancer could also be considered for metronomic chemotherapy applications. While a metronomic combination of CPA and methotrexate is being studied in the adjuvant setting involving patients with ER- and PR-negative breast cancer (IBCSG 22-00, www.ibcsg.org), no such studies are yet underway for locally advanced prostate cancer following definite local therapy. Interestingly, adjuvant androgen deprivation therapy (ADT), the standard therapy in this setting, seems to act through antiangiogenic mechanisms (Nicholson and Theodorescu 2004). Thus, a strategy of combined ADT plus metronomic chemotherapy might be an interesting alternative to other approaches currently being studied which involve MTD chemotherapy (Glode 2006). For similar reasons, metronomic chemotherapy might also become an option for the treatment of hormone-sensitive prostate cancer, either concomitant with ADT or sequentially (in ADT-free intervals) when intermittent ADT is applied.

10.4 Conclusions and Perspectives

Over the last few years, beneficial effects of antiangiogenic tumor therapies have been described in several tumor types (Ferrara and Kerbel 2005). It also became increasingly clear that by changing the way of administration, the primary cellular target of cytotoxic drugs can shift from the tumor cell population to the tumor neovasculature, representing a potent antiangiogenic treatment approach. Metronomic chemotherapy is unlikely to replace conventional MTD chemotherapy administration when rapid tumor cell killing is needed. However, given the particular mode of action and the beneficial

safety profile, it is likely to become a valuable alternative in combination therapies involving targeted (antiangiogenic) agents and in the palliative setting. Because many aspects of the metronomic approach remain empirical, major efforts are still needed to bring this novel and emerging concept to full fruition. Furthermore, the long-term administration of oral drugs involves new challenges such as treatment adherence and possibly an increased risk of interference with comedications (Emmenegger et al. 2007). Despite these drawbacks, metronomic chemotherapy has already come a long way from its description less than 10 years ago. It is hoped that the exciting Phase II trial results will be confirmed in future Phase III trials.

Acknowledgments The work summarized in this review was supported by grants from the National Cancer Institute of Canada, the Canadian Institutes for Health Research, and the National Institutes of Health, USA, to Robert S. Kerbel, and by sponsored research agreements with ImClone Systems, New York, and Taiho Pharmaceuticals, Japan. Urban Emmenegger is supported by the Ontario Institute for Cancer Research through funding provided by the Province of Ontario. We thank Cassandra Cheng for her excellent secretarial assistance.

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