

# Metronomic Chemotherapy: The Hype and the Science

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The survival benefits of conventional maximum tolerated dose (MTD) chemotherapy are modest for the treatment of many canine and feline cancers. In addition, conventional chemotherapy may induce significant acute and chronic toxicities. Recent mouse studies have demonstrated that metronomic chemotherapy, which is defined as the uninterrupted administration of low doses of cytotoxic drugs at regular and frequent intervals, could be at least as effective as MTD therapy and associated with substantially less toxicity and expense. In comparison to conventional MTD chemotherapy, the critical difference of metronomic therapy appears to be the elimination of long break periods between treatments. Elimination of treatment gaps also eliminates, or at least substantially reduces, the ability of tumor cells to undergo repopulation and damage repair and to alter their microenvironment. Even in the setting of drug-resistant disease, metronomic dosing protocols can occasionally elicit significant and durable treatment responses in people with heavily pre-treated, metastatic malignancies.

## How metronomic chemotherapy works

In contrast to conventional chemotherapy protocols, which target rapidly dividing tumor cells, a key target of metronomic chemotherapy is the tumor vasculature. Tumor angiogenesis is thought to occur at two levels in the cancer patient: (1) locally within the tumor microenvironment, where local endothelial cells (ECs) are stimulated to proliferate and (2) via systemic effects on bone marrow-derived circulating endothelial progenitor cells (CEPs). Continuous administration of low doses of a surprisingly wide range of drugs (such as cyclophosphamide, methotrexate, vinblastine and paclitaxel) is cytotoxic to both growing ECs and CEPs but has no effect on non-endothelial cells such as tumor cells, epithelial cells and leukocytes. Because of their relative genetic stability, ECs are inherently less susceptible to the development of drug resistance than are tumor cells. Even when maximally tolerated doses of chemotherapy drugs are no longer effective, significant inhibition of tumor growth and sparing of normal tissue can sometimes be achieved by simply changing to a metronomic dosing regimen.

Another target of metronomic chemotherapy is the regulatory T cell ( $T_{reg}$ ), a subset of the CD4<sup>+</sup> T-lymphocyte population that facilitates tumor survival in cancer patients through immunosuppressive effects. The selective toxicity of metronomic chemotherapy for  $T_{reg}$  has been best studied for the alkylating agent cyclophosphamide (CYC). In mice and in humans, administration of low doses of CYC leads to a transient but profound decrease in the number of circulating  $T_{reg}$  and directly inhibits  $T_{reg}$  function. Several recent studies show that it may also be possible to decrease  $T_{reg}$  through the administration of cyclooxygenase (COX) inhibitors. For example, administration of COX inhibitors to mice with lung cancer resulted in suppression of  $T_{reg}$  function and a decrease  $T_{reg}$  numbers, in part through inhibition of tumor-induced expression of COX-2 and prostaglandin E2. Effects of low dose CYC on dendritic cells have also been reported.

## The application of metronomic chemotherapy to veterinary patients

Although the results of many clinical trials investigating metronomic chemotherapy in human cancer patients appear promising, a significant limitation has been considerable empiricism in the establishment of optimal metronomic dosing schedules and in evaluating therapeutic activity. Several studies have recently shown, however, that the optimal biologic dose of metronomic chemotherapy (defined as the dose eliciting maximum reduction in tumor volume without inducing significant systemic toxicity) corresponds closely to the reduction in CEP levels in the blood. EC numbers also appear to correlate well with other standard measures of tumor angiogenesis such as tumor microvessel density (MVD) assessment. CEP have tentatively been identified in dogs using flow cytometry and, as in people, may be useful in helping to identify optimal drug doses and schedules for metronomic regimens. Although  $T_{reg}$  levels are known to decrease in human patients receiving low doses of CYC, the validity of using  $T_{reg}$  numbers to monitor metronomic dosing protocols awaits further investigation.

To date, the application of metronomic chemotherapy to veterinary patients has been based on anecdotal reports and extrapolation from human studies. Evaluation of metronomic drug dosing has recently been described in several small clinical trials (**Table 1**). In one of these studies, dogs with splenic hemangiosarcoma received a combination of 3 drugs (CYC, etoposide and piroxicam). Study design did not permit determination of single drug efficacy or optimal drug dosing, nor were tumor biomarkers assessed. In the other study, a significant treatment effect from administration of low-dose continuous CYC and piroxicam therapy was described in dogs with incompletely resected soft tissue sarcoma. As in the canine hemangiosarcoma trial, the relative contribution of each drug could not be evaluated and drug dosages were empirically determined based on human clinical trials. Several clinical studies have recently described tolerability of metronomic dosing of chlorambucil and lomustine in tumor-bearing dogs; assessment of antiangiogenic markers to determine drug dosages were not reported, although some antitumor effects were reported.

**Table 1. Published clinical trials of metronomic chemotherapy in dogs**

Agent	Dosage	Concurrent Drugs	Comments	Reference
CYC	12.5-25 mg/m <sup>2</sup> /d	Piroxicam, etoposide	Only given 50% of the time, dosage empiric	Lana 2007
CYC	10 mg/m <sup>2</sup> /d	Piroxicam	Dosage empiric	Elmslie 2008
CYC	10-17.5 mg/m <sup>2</sup> /d		15-17.5 mg/m <sup>2</sup> identified as optimal	Burton 2011
CYC	15 mg/m <sup>2</sup> /d	Toceranib	Combination was well tolerated, possible additive Treg depletion	Mitchell 2012
Lomustine	2.84 mg/m <sup>2</sup> /d		Dosage empiric. >25% of dogs discontinued d/t toxicity. ORR = 6%	Tripp 2011
Chlorambucil	4 mg/m <sup>2</sup> /d		Dosage empiric. ORR = 11%	Leach 2012

CYC = cyclophosphamide

ORR = overall response rate

### How do we know if metronomic chemotherapy is working the way we think it is?

The low risk for toxicity, reasonable cost, ease of administration and potential for significant therapeutic benefit makes metronomic chemotherapy a particularly attractive treatment option for companion animals with cancer. There is clearly a need, however, to conduct basic studies that enable the identification of effective protocols using relevant tumor biomarkers in addition to measuring clinical parameters such as disease-free interval and survival time. Towards this end, we investigated the effectiveness of metronomic dosing of single-agent oral CYC in dogs with soft tissue sarcoma (STS) to begin to identify the effects of individual agents in these multi-drug protocols. The primary goals of this trial were to determine whether low-dose CYC therapy decreased T<sub>reg</sub> and/or exhibited antiangiogenic activity.

### Low-dose cyc decreases treg and tumor microvessel density in dogs with soft tissue sarcoma

To determine the effects of metronomic CYC therapy on numbers of T<sub>reg</sub> in circulation, blood samples were collected from client-owned dogs with measurable grade I or II soft tissue sarcoma on days 0, 14, and 28 after beginning oral CYC. Two cohorts were evaluated based on preliminary studies, with one group of dogs receiving daily oral CYC at a target dosage of 12.5 mg/m<sup>2</sup> daily and the other at a target dosage of 15 mg/m<sup>2</sup> daily. Dogs receiving CYC at a target dosage of 15.0 mg/m<sup>2</sup>/day had significant decreases in both the absolute number and percentage of T<sub>reg</sub> in the peripheral blood during the 28-day study period. Despite the decrease of T<sub>reg</sub> at the higher dosage of CYC, there were no changes in the absolute numbers or percentages of either CD4<sup>+</sup> or CD8<sup>+</sup> lymphocytes during the course of the clinical trial, suggesting that the effects of CYC were selective for the T<sub>reg</sub> subset of T-lymphocytes.

Tumor MVD was also assessed in these patients using immunohistochemistry. There were no significant changes in MVD between the three study time points in the tumors of dogs treated with 12.5 mg/m<sup>2</sup>/day CYC. However, in the dogs receiving CYC at 15.0 mg/m<sup>2</sup>/day, the MVD significantly decreased from the pretreatment mean. This decrease persisted throughout the 28-day study period and was also significantly decreased from the MVD prior to therapy. Together, these results suggest that both antiangiogenic and immunomodulatory effects of CYC occur when administered at 15 mg/m<sup>2</sup>/day.

### Conclusions and future directions

Although this small study provides a starting point for metronomic dosing of CYC, many factors such as the pharmacokinetics of low-dose CYC therapy and effects of drug combinations await investigation. It is also not clear whether changes in circulating numbers of T<sub>reg</sub> corresponds to antiangiogenic effects for drugs other than CYC or whether T<sub>reg</sub> analysis could serve as a surrogate for tumor MVD and CEC assessment. At this time, assessment of canine T<sub>reg</sub> and particularly of CECs remains technically challenging; neither assay is available for widespread use. Other potential biomarkers for antiangiogenic therapy, such as gene expression of VEGFR-2 and VE-Cadherin in peripheral blood, are also being evaluated in human patients and may be applicable to monitoring of veterinary patients as well. In conclusion, more clinical research on safety, efficacy and validation of surrogate markers of angiogenesis should be performed before the widespread use of chemotherapy drugs in a metronomic schedule makes its way into standard oncology practice.

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