Pharmacokinetics and Pharmacodynamics-Based Mathematical Modeling Identifies an Optimal Protocol for Metronomic Chemotherapy

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Abstract

Metronomic chemotherapy is usually associated with better tolerance than conventional chemotherapy, and encouraging response rates have been reported in various settings. However, clinical development of metronomic chemotherapy has been hampered by a number of limitations, including the vagueness of its definition and the resulting empiricism in protocol design. In this study, we developed a pharmacokinetic/pharmacodynamic mathematical model that identifies in silico the most effective administration schedule for gemcitabine monotherapy. This model is based upon four biological assumptions regarding the mechanisms of action of metronomic chemotherapy, resulting in a set of 6 minimally parameterized differential equations. Simulations identified daily 0.5–1 mg/kg gemcitabine as an optimal protocol to maximize antitumor efficacy. Both metronomic protocols (0.5 and 1 mg/kg/day for 28 days) were evaluated in chemoresistant neuroblastoma-bearing mice and compared with the standard MTD protocol (100 mg/kg once a week for 4 weeks). Systemic exposure to gemcitabine was 14 times lower in the metronomic groups compared with the standard group. Despite this, metronomic gemcitabine significantly inhibited tumor angiogenesis and reduced tumor perfusion and inflammation in vivo, while standard gemcitabine did not. Furthermore, metronomic gemcitabine yielded a 40%–50% decrease in tumor mass at the end of treatment as compared with control mice (P = 0.002, ANOVA on ranks with Dunn test), while standard gemcitabine failed to significantly reduce tumor growth. Stable disease was maintained in the metronomic groups for up to 2 months after treatment completion (67%–72% reduction in tumor growth at study conclusion, P < 0.001; ANOVA on ranks with Dunn test). Collectively, our results confirmed the superiority of metronomic protocols in chemoresistant tumors in vivo. Cancer Res; 77(17): 4723–33. ©2017 AACR.

Major Finding

Our study demonstrates the potential of pharmacokinetic/pharmacodynamic mathematical modeling to optimize metronomic chemotherapy protocols, which can be tested in nonclinical models and future clinical trials.

Introduction

Metronomic chemotherapy (MC) is defined as the chronic administration of chemotherapeutic agents at relatively low, minimally toxic doses, and with no prolonged drug-free breaks (1). Such regimen was originally thought to act mainly through inhibition of tumor angiogenesis (2). However, additional mechanisms have been unveiled, including immunomodulatory effects and impairment of cancer stem cells, making MC a prime example of multi-targeted therapy (3). Numerous clinical trials have been conducted to evaluate the potential of metronomic protocols in a variety of settings, both in adults and in children, with some encouraging results (4, 5). MC is usually associated with improved tolerance, as compared with standard regimens based upon the MTD paradigm, making it a particularly attractive alternative in frail patients and to be associated with targeted therapies or immune checkpoint inhibitors.

However, the definition of MC remains ambiguous and raises a number of critical questions regarding what is a chronic administration (e.g., weekly, twice a week, thrice a week, daily…) or a "relatively low dose" (e.g., half, one third, one tenth of the MTD…). As such, even for a single drug to be administered in a given setting, a multitude of modalities exists and would all require to be tested to define an optimal protocol. This holds true for cyclophosphamide, capcitabine, gemcitabine, temozolomide, or vinorelbine, to name a few chemotherapy agents commonly used in metronomic protocols (6–8). Therefore, MC...
The four following assumptions were made prior to developing our mathematical model:

(i) Tumor growth follows a Gompertz model (9).
(ii) Endothelial cells are more sensitive to chemotherapy agents than cancer cells (10).
(iii) Depleting endothelial cells will affect tumor growth (11).
(iv) Endothelial cells are more genetically stable than cancer cells and therefore less likely to develop resistance to chemotherapy agents.

Several studies have indeed reported that tumor-bearing mice, which did not respond or acquired resistance to a cytotoxic agent when administered at or near the MTD, could still respond to the same agent given metronomically (i.e., administered at lower dose but more frequently; refs. 12, 13).

Our structural mathematical model is deliberately phenomenological to involve the minimum number of parameters, and equations only address biological processes that are considered to be predominant in metronomic chemotherapy. This model is built upon our previous model for the administration of temozolomide (14) and adapted to the administration of gemcitabine. To parameterize our model, we used previously published pharmacokinetic/pharmacodynamic data from the Peters and colleagues’ study (15–17) and Monolix software. The model is described by a set of six differential equations:

\[
\begin{align*}
\frac{\text{d}N_1}{\text{d}t} &= -\frac{C}{C_0} \cdot y_1 + \frac{C}{C_0} y_2, \\
\frac{\text{d}y_1}{\text{d}t} &= -a_1 \exp(-b_1 y_2) + (y_1 - c_1) H(y_1 - c_1), \\
\frac{\text{d}y_2}{\text{d}t} &= -a_2 \exp(-b_2 y_1) + (y_2 - c_2) H(y_2 - c_2), \\
\frac{\text{d}y_3}{\text{d}t} &= \lambda y_3 \log\left(\frac{y_2}{y_4}\right) - N_1 y_4, \\
\frac{\text{d}y_4}{\text{d}t} &= R - (R + N_2) y_5, \\
\frac{\text{d}y_5}{\text{d}t} &= (y_1 - c_1) H(y_1 - c_1).
\end{align*}
\]

With \(N_1 = \exp(-\text{res} \cdot y_k) \cdot u_1 \cdot y_2\), where \(\text{res}\) and \(u_1\) are constants and \(N_2 = u_2 y_b\), where \(u_2\) is a constant.

Equation A refers to the pharmacokinetics of gemcitabine, which have been shown to be best described by a one-compartment model (17). Equations B and C, which are based on an interface model recently described (18), reflect the drug effect on tumor and endothelial cells, respectively. The parameters \(a_1\) and \(a_2\) involved in these equations can be seen as impact parameters on the two cell types. Interestingly, endothelial cells are shown to be more sensitive to chemotherapy than tumor cells, as confirmed by in vitro experiments showing that the EC50 of gemcitabine was 527 times lower in endothelial cells than in neuroblastoma cells (Supplementary Fig. S1). This higher sensitivity of endothelial cells compared with tumor cells will result in much higher values of \(a_1\) as compared with \(a_2\) and minimum effective concentrations for tumor and endothelial cells, denoted \(c_1\) and \(c_2\), also checking \(c_1 > c_2\). Equation D describes the evolution of the number of tumor cells using a modified Gompertz model to take into account not only the antitumor cytotoxic effect of gemcitabine (by the term \(-N_1 y_4\)) but also the slowing down of tumor growth due to decreased blood supply, a consequence of the destruction of the endothelial compartment, by the term \(\lambda y_3\) that replaces the \(\lambda\) constant of the classical Gompertz model. Equation E describes the evolution of endothelial cell number based on their destruction by gemcitabine (by the term \(-N_2 y_5\)). Finally, equation F reflects the area under the curve to quantify drug exposure and models the emergence of resistant cells over time (by the term \(N_1 = \exp(-\text{res} \cdot y_k) \cdot u_1 \cdot y_2\)]. The more resistant cells develop \(y_5\), the smaller the antitumor cytotoxic effect \(N_1\) will be.

protocols used in the clinic to date are exclusively empirical in terms of dosing and scheduling (4, 6).

Determining an optimal metronomic regimen among numerous possibilities by empirical research (i.e., trial-and-error testing) is not only time- and resource-consuming, but also underpowered. In this respect, pharmacokinetic/pharmacodynamic analysis and mathematical modeling can bring a valuable step forward by reducing the number of parameters and therefore underpowered possibilities by empirical research (i.e., trial-and-error testing) is not only time- and resource-consuming, but also underpowered. In this respect, pharmacokinetic/pharmacodynamic analysis and mathematical modeling can bring a valuable step forward by reducing the number of parameters and therefore underpowered possibilities by empirical research (i.e., trial-and-error testing) is not only time- and resource-consuming, but also underpowered. In this respect, pharmacokinetic/pharmacodynamic analysis and mathematical modeling can bring a valuable step forward by reducing the number of parameters and therefore underpowered possibilities by empirical research (i.e., trial-and-error testing).
and to demonstrate in vivo the predicted superiority of these protocols over the standard regimen in a mouse model of chemoresistant neuroblastoma.

Materials and Methods

Model equations

As explained in the quick guide to equations, our model is described by a set of six differential equations:

\[
\begin{align*}
\frac{d y_1}{dt} &= \frac{C_l}{V} y_1 + u(t) \\
\frac{d y_2}{dt} &= -a_1 \exp(-b_1 y_2) y_2 + (y_1 - c_1) H(y_1 - c_1) \\
\frac{d y_3}{dt} &= -a_2 \exp(-b_2 y_3) y_3 + (y_1 - c_2) H(y_1 - c_2) \\
\frac{d y_4}{dt} &= \frac{\lambda y_5}{y_5} \log \left( \frac{K}{y_4} \right) - N_1 y_4 \\
\frac{d y_5}{dt} &= R - (R + N_2) y_5 \\
\frac{d y_6}{dt} &= (y_1 - c_1) H(y_1 - c_1)
\end{align*}
\]

(a) \( y_1 \): pharmacokinetic modeling of gemcitabine, with \( C_l \) representing clearance, \( V \) the volume of distribution, and \( u(t) \) the perfusion rate.

(b) \( y_2, y_1 \): effect of gemcitabine on cancer cells (for \( y_2 \)) and on endothelial cells (for \( y_1 \)). The parameters \( a_1 \) and \( a_2 \) can be seen as impact parameters on the two cell types, and the constant \( c_1 \) and \( c_2 \) represent the minimum effective concentrations for tumor and endothelial cells, respectively. Given the higher chemosensitivity of endothelial cells as compared with cancer cells, the parameters of this equation check \( a_1 > a_2 \) and \( c_1 < c_2 \).

(c) \( y_4 \): tumor growth modeled by a modified Gompertz function (1).

(d) \( y_5 \): antiangiogenic effect of gemcitabine. The baseline value \( 1 \), for no antiangiogenic effect. An antiangiogenic effect is present when \( y_5(t) < 1 \).

(e) \( y_6 \): area under the curve of plasmatic concentration \( y_1 \) with a threshold (AUC).

The pharmacokinetics of gemcitabine was best described by a one-compartment model (17). Intraperitoneal administration was taken into account by introducing a lag-time of 5 minutes to express the delay to reach the systemic circulation. The pharmacodynamics model involved a double effect on tumor cells and endothelial cells, and takes into account acquired resistance in tumor cells. Finally, a delay was introduced by a chain of four transit compartments in \( N_t \) to reproduce the delay observed in tumor shrinkage (24).

Cell culture

The human neuroblastoma cell line, GI-ME-N, originally established at relapse, was purchased from Leibniz-Institut DSMZ (Germany) in 2014 and maintained in culture in RPMI supplemented with 10% FCS and 5% CO2. This cell line was authenticated by the manufacturer through fluorescent nonaplex PCR of short tandem repeat markers. Upon reception, master stocks were prepared and frozen in liquid nitrogen. Subsequently, cells were not kept in culture for more than 2 months, they were routinely tested and were negative for mycoplasma contamination. These cells display aberrant promoter methylation of p14ARF, which confers chemoresistance (25). They were stably infected with Red-Fluc-Puromycin lentivirus, under the control of the UBC promoter (Perkin Elmer). The firefly luciferase transgene is fused to the puromycin resistance gene via a self-cleaving linker peptide for coexpression with selection marker. Transfected cells were selected with puromycin following manufacturer’s recommendations, and maintained in standard conditions (i.e., RPMI supplemented with 10% FCS, plus 3.5 μg/mL maintenance of puromycin). Bioluminescence was assessed by seeding an increasing number of previously selected GI-ME-N-Luc+ cells (i.e., 10, 100, 1,000, 10,000, 100,000, 106) in 6-well plates supplemented with 1 μg/mL luciferin (Perkin Elmer) immediately before bioluminescence signal acquisition using a Spectrum IVIS imager and Living Image 4.2. Software (Perkin Elmer). A linear relationship \( (r^2=0.999) \) between the number of cells and the bioluminescence signal was found, with each cell emitting an average 4,203 photons per second (Supplementary Fig. S2). Resulting GI-ME-N-Luc+ clones were characterized (i.e., cell morphology, doubling time, in vitro sensitivity to gemcitabine) to ensure that they did not differ from the parental GI-ME-N cells.

Animal model

All experiments were submitted and approved by the animal ethics committee of Aix Marseille University (CE14) and the French Minister of "L’Education Nationale, l’Enseignement Supérieur et la Recherche (MENESR)" prior to starting the experiments and the study was registered under the #02150.01 MENESR identification number. Experiments were conducted in compliance with European regulations, based on the UKCCCR guidelines for the welfare of animals in experimental oncology (26). Four-week-old female Swiss nude mice (Charles River Laboratories) were subcutaneously engrafted on the left flank with 80,000 GI-ME-N-Luc+ cells in 60% Matrigel (Corning). Mice (\( n = 12 \) per group) were then randomized into four different groups: control (saline intraperitoneal injection once a week for 4 consecutive weeks), standard gemcitabine (100 mg/kg i.p. once a week for 4 consecutive weeks), MetroGem-1 (1 mg/kg/day for 28 continuous days) and MetroGem-0.5 (0.5 mg/kg/day for 28 continuous days). For both metronomic groups, gemcitabine was administrated subcutaneously using an osmotic pump (Alzet) filled with gemcitabine solutions calibrated for delivering the required dosing on a daily basis. During all experiments, mice were maintained in sterilized filter-stopped cages kept in a sterile and thermostated cabinet. They were monitored daily for signs of distress, decreased physical activity, or any behavioral change. Water was supplemented with paracetamol (eq. 80 mg/kg/day) to prevent metastatic disease-related pain (27). Animals were euthanized under anesthesia when showing signs of distress, pain, cachexia (i.e., loss of >15% of body weight), or when tumor reached 2 cm3, as estimated by caliper measurement using the Carlsson formula (28).

Bioluminescence imaging

Animals were imaged twice-a-week for tumor growth. Briefly, mice were injected intraperitoneally with 150 mg/kg luciferin, 12 minutes before being imaged using the Spectrum IVIS system (Perkin Elmer). Resulting signals were acquired and ROI calculations performed using the Living Image 4.2. Software. Final results were expressed as photons/second. In addition, whole-body 3D bioluminescence imaging was undertaken to look for metastasis at treatment start and on days 15 and 30 by intraperitoneal
injection of 200 mg/kg luciferin. To generate 3D bioluminescence signals, images were acquired between 560 and 680 nm emission wavelengths with a 20 nm step. Imaging, DLIT reconstruction, and data processing were next performed using the Living Image 4.2 software.

Drug monitoring

Plasma levels of gemcitabine and its main metabolite, difluoro-deoxy-uridine (dFdU), were evaluated in satellite subgroups (n = 3) taken from the standard gemcitabine and MetroGem-1 groups on blood sample withdrawn by intracardiac puncture in tetrahydro-uridine–coated heparin tubes. For mice undergoing standard gemcitabine treatment, blood samples (200 μL) were taken immediately after drug injection. For mice in the MetroGem-1 group, blood samples were collected at steady state. After centrifugation, plasma was isolated and samples were stored at −80°C until analysis. Gemcitabine and dFdU metabolite were quantitated by LC/MS-MS following a validated method derived from Marangon and colleagues (29). Respective gemcitabine AUCs were calculated by noncompartmental analysis, with an estimated theoretical mean total plasma clearance of gemcitabine of 0.55 ml/g/hour (30).

Vascular density and tumor blood flow monitoring

Mice from satellite subgroups (n = 3 mice per condition) taken from the standard gemcitabine and MetroGem-1 groups were injected intravenously (caudal vein) with 50 mL of AngioSense 750 (Perkin Elmer) for fluorescence imaging of blood vessels and vascular density, and Integrisense 750 (Perkin Elmer, France) for fluorescence imaging of αvβ3 integrin in both tumor cells and the neovasculature at treatment conclusion. Briefly, 24 hours after injection, mice were imaged at 680 nm using the Spectrum IVIS system. Resulting signals were acquired and ROI calculations performed and treated using the Living Image 4.2 software. Final results were expressed as photons/second.

Results

Calibration of mathematical model parameters

The parameters of our mathematical model were estimated from experimental data published in pivotal articles by Peters and colleagues (15–17). Data regarding the pharmacokinetic profiles and pharmacodynamic endpoints (i.e., reduction in tumor growth) of gemcitabine administered in tumor-bearing mice following a variety of dosing and schedules were used. Model parameters were estimated to achieve the best fit between simulated and experimental curves using a maximum likelihood minimization function implemented in Monolix software. Pharmacokinetic parameters were estimated using Monolix 4.3.3 on digitalized pharmacokinetic data and pharmacodynamic model parameters were calibrated to describe the four profiles available (Fig. 1; Supplementary Fig. S3).
Estimated values for the different parameters are summarized in Table 1. In particular, we can note that the estimates found for $a_1 (= 445)$ and $a_2 (= 0.155)$ have orders of magnitude consistent with those independently observed in vitro (Supplementary Fig. S1).

Model predictions

Once the model has been calibrated and parameterized for gemcitabine (Table 1), simulations of different dosing regimens were performed. First, we simulated the administration of gemcitabine at the MTD, that is, 100 mg/kg every week for four administrations (Fig. 2). Our model predicts that this treatment regimen initially controls tumor progression but this effect is only transient and tumor growth resumes after approximately 20 days of treatment. We then changed the schedule of administration while maintaining the cumulative dose of gemcitabine and thus tested the impact of 50 mg/kg twice a week, 20 mg/kg five days a week, and 10 mg/kg every day. Our simulations suggest that increasing the frequency of treatment will dramatically increase antitumor efficacy. However, although highly efficacious in silico, this type of dose-dense chemotherapy regimen may lead to increased toxicity. To overcome this issue, we applied our model to identify the lowest effective concentration of gemcitabine when administered daily (Fig. 3). Our simulations show that while 0.1 mg/kg and 0.2 mg/kg were inefficient, long-term control of tumor progression was obtained with 0.5 mg/kg and 1 mg/kg, with little to no tumor regrowth after 28 days of treatment.

In vivo validation – pharmacokinetic monitoring

On the basis of our model predictions, we decided to evaluate the antitumor efficacy of three different treatment regimens in neuroblastoma-bearing mice: a nonoptimal MTD regimen based on weekly intravenous injections of gemcitabine at 100 mg/kg for 4 consecutive weeks (StandardGEM group) and 2 optimal low-dose regimens based on continuous subcutaneous infusion of gemcitabine at 1 and 0.5 mg/kg per day for 4 consecutive weeks (MetroGem-1 and MetroGem-0.5 groups). All three regimens were well tolerated as no significant change in body weight was found between the control and treatment groups during this study (Supplementary Fig. S4). Drug monitoring showed that mean peak plasma concentrations of gemcitabine were 63.839 ± 5.428 ng/mL in the StandardGEM group and 108 ± 25 ng/mL for the MetroGem-1 group (Fig. 4). Mean concentrations in dFdU metabolite were 34.0 ± 1.6 ng/mL in the StandardGEM group and 4.2 ± 1.6 ng/mL for the MetroGem-1 group but were not necessarily representative of their respective $C_{\text{max}}$. Simulated cumulative AUCs for gemcitabine calculated by noncompartimental analysis were 10 mg/mL/min for StandardGEM and 0.69 mg/mL/min for MetroGem-1 over 28 continuous days. The 1 mg/kg/day metronomic schedule thus led to a 14-time lower systemic exposure as compared with standard MTD treatment, which is consistent with the differences in cumulative doses administered (100 mg/kg per week versus 7 mg/kg per week).

In vivo validation – impact on tumor angiogenesis and inflammation

Impact of treatments on vascular density and tumor blood perfusion was evaluated by bioluminescence and fluorescence imaging (Fig. 5). Monitoring the time to reach the plateau in bioluminescence signal first showed marked differences in slopes between treatment groups (Fig. 5A). Resulting slope of the time-to-plateau was 5.4 and 3.2 times lower in metronomically treated mice as compared with control and StandardGEM mice, respectively (2.5 ± 1.1 vs. 13.4 ± 2.4 and 8.1 ± 2.2; $P < 0.01$, one-way ANOVA with Student Keuls multiple comparison testing). This suggested a decrease in tumor blood perfusion in the MetroGem-1 group as compared with control and StandardGEM groups. Angiosense imaging further confirmed this finding (Fig. 5B) and showed that the MetroGem-1 regimen led to a statistically significant 69% reduction in vascular density as compared with control group (1.5 × 10^9 ± 1.1 × 10^9 γ/second vs. 4.9 × 10^9 ± 6.0 × 10^8 γ/second; $P = 0.014$, one-way ANOVA with Newmann–Keuls multiple comparison testing), whereas a nonsignificant 34% increase was observed in mice treated with standard gemcitabine (6.5 × 10^9 ± 1.9 × 10^9 γ/second vs. 4.9 × 10^9 ± 6.0 × 10^8 γ/second). Similar results were obtained when comparing integrin αvβ3 signals between treatment groups (Fig. 5C) although the differences were not statistically significant (control: 1.7 × 10^10 ± 1.1 × 10^10 γ/second; StandardGEM: 1.6 × 10^10 ± 2.5 × 10^9 γ/second; MetroGem-1: 1.3 × 10^10 ± 1.1 × 10^9 γ/second; $P = 0.054$, one-way ANOVA with Newmann–Keuls multiple comparison testing). Finally, cysteine cathepsin proteinase activity between metronomically treated mice (7.8 × 10^10 ± 4.5 × 10^10 γ/second) and both control mice (1.1 × 10^11 ± 4.1 γ/second) and mice treated with standard gemcitabine (9.95 × 10^10 ± 0.05 × 10^10 γ/second; $P < 0.0005$; one-way ANOVA with Newmann–Keuls multiple comparison testing).

In vivo validation – impact on tumor growth

Bioluminescence monitoring of tumor growth is shown in Fig. 6. Both metronomic schedules led to a significant 40.5% (MetroGem-1) and 49% (MetroGem-0.5) reduction in mean tumor mass after 28 days of treatment as compared with control group, whereas standard gemcitabine failed to reduce tumor growth (+28%; $P > 0.05$). Tumor masses expressed as emitted photons per second (γ/sec) were 1.95 × 10^8 ± 6.8 × 10^7 (control group), 2.5 × 10^8 ± 6.5 × 10^7 (StandardGEM), 1.16 × 10^8 ± 0.6 × 10^7 (MetroGem-1) and 9.9 × 10^7 ± 4.0 × 10^7 (MetroGem-0.5), respectively. Microvessel density (CD31 staining) was significantly reduced in mice treated with the two metronomic regimens (1.1 ± 0.1 vs. 1.1 ± 0.1; $P < 0.01$, one-way ANOVA with Student Keuls multiple comparison testing). A significant decrease in integrin αvβ3 levels were noted in mice treated with the two metronomic regimens (1.1 ± 0.1 vs. 1.1 ± 0.1; $P < 0.01$, one-way ANOVA with Student Keuls multiple comparison testing) and the resulting inhibition was associated with a significant increase in the accumulation of lipofuscin-like fluorescence (1.1 ± 0.1 vs. 1.1 ± 0.1; $P < 0.01$, one-way ANOVA with Student Keuls multiple comparison testing).
Because Equal Variance testing failed, ANOVA on ranks was performed instead of one-way ANOVA. A statistically significant difference was found between the groups ($P = 0.002$) and further Dunn test identified groups treated with metronomic regimens as significantly different from control and StandardGEM treatment, whereas no difference was found between StandardGEM and control groups. Interestingly, tumor growth was monitored up to 2 months after the end of the treatment and showed that stable disease was maintained in the metronomic groups until study conclusion, whereas tumors kept growing in control and StandardGEM groups. At study conclusion (i.e., 97 days after starting the experiment), mean tumor mass expressed as bioluminescent signal were $5.2 \times 10^8$ $/C_{210^8}/C_{6}$, $5.4 \times 10^8$ $/C_{210^8}/C_{6}$, $1.75 \times 10^8$ $/C_{210^8}/C_{6}$, and $1.73 \times 10^8$ $/C_{210^8}/C_{6}$ (control group), and $8.6 \times 10^7$ $/C_{210^7}$ (StandardGEM), $1.7 \times 10^7$ $/C_{210^7}$ (MetroGEM-1), and $4.7 \times 10^7$ $/C_{210^7}$ (MetroGEM-0.5). A significant reduction in tumor mass by 67% (MetroGEM-1) and 72% (MetroGEM-0.5) was therefore found in metronomic groups as compared with control or standard gemcitabine groups ($P < 0.001$; ANOVA on ranks with Dunn test). Extensive three-dimensional imaging of whole body to search for secondary lesions was not conclusive because no metastasis was evidenced with GI-ME-N-Luc+ xenografts in nude mice (data not shown).

**Discussion**

Since its inception in 2000, MC has been extensively studied in preclinical cancer models and investigated in more than 150 clinical trials to date (3, 4, 20). These trials showed promising results in some hard-to-treat cancers and in specific settings, such as patients with poor performance status or patients living...
in low- and middle-income countries (reviewed in ref. 32). However, MC is yet to be widely accepted in general clinical practice in oncology. This discrepancy between encouraging results in clinical trials and delayed clinical acceptance is due to multiple factors, including limited number of completed phase III trials, lack of reliable biomarkers, ambiguity of terminology, and

![Figure 3.](image)

*In silico* simulations of antitumor efficacy for daily administration of gemcitabine at different doses. Model predictions of pharmacokinetics (left) and antitumor efficacy (right) of gemcitabine following different daily administration regimens. *X*-axis represents the time in hours, while *y*-axis represents the plasma concentration of gemcitabine in ng/mL (left) or the tumor burden in arbitrary unit (right).

![Figure 4.](image)

Gemcitabine plasma concentration measured *in vivo*. Plasma levels of gemcitabine (A) and its main metabolite (B), di-fluoro-deoxy-uridine (dFdU), were evaluated by LC/MS-MS in satellite subgroups (*n* = 3) from the standard gemcitabine and MetroGEM-1 cohorts. For StandardGEM mice, blood samples were taken immediately after drug injection, while they were collected at steady state in mice from the MetroGEM-1 group. Logarithmic scale for *y*-axis.
empiric design of treatment protocols. To address the latter, we developed a mathematical pharmacokinetic/pharmacodynamic model to identify the most effective metronomic protocol among a wide variety of possibilities, and validated the predictions of computer simulations by *in vivo* experiments.

Gemcitabine is a potential drug to be added in the therapeutic armamentarium against neuroblastoma, a devastating form of childhood cancer responsible for 15% of all cancer-related deaths in children. While its antitumor activity is usually limited when given as a single agent (33, 34), it seems promising in...
combination with other cytotoxics (35, 36) or with antiangiogenic drugs (37). The fact that antiangiogenics could be effective in neuroblastoma suggests that metronomic administration of gemcitabine, a regimen known to yield additional antiangiogenic effects (38, 39), could be of interest to treat this pathology. In addition, metronomic gemcitabine may also deplete myeloid-derived suppressor cells and thus contribute to restoring some antitumor activity of the immune system (40, 41). This is particularly relevant in neuroblastoma given the recent success of the combination of anti-GD2 antibody with IL2 therapy in this disease (42).

Several studies have investigated the therapeutic potential of metronomic gemcitabine in a variety of cancer models such as liver, pancreas, or head and neck tumors. In these studies, treatment schedule covered numerous modalities, such as intermittent 30 mg/kg on days 1, 4, 7, 10, 13, 16, 19, 22, 25, and 28 (43), intermittent 25 mg/kg on days 1, 3, 5 for 3 weeks (44), intermittent 15 mg/kg on days 5, 9, 12, 16, 15, continuous 3.3 mg/kg/day for 21 days (46), or continuous 1 mg/kg/day for 28 days (38, 47, 48), to name a few. Of note, the antitumor effect of gemcitabine is known to be highly dependent upon the administration schedule and early studies have suggested that MTD and intermittent dosing could be more effective than daily administration at lower doses (15).

Owing to the rising complexity when designing combined strategies in cancer, the time for trial-and-error practices is coming to an end and there is an urgent need for developing tools likely to rationalize the use of anticancer agents. For instance, metronomic regimen is a global, generic term encompassing a wide range of possibilities in terms of doses and schedules. Developing computational pharmacology approaches in oncology is therefore a rising trend. Of note, most strategies undertaken to date rely on the development of highly sophisticated mechanistic models to describe tumor biology and/or pharmacologic effects, in a multiparameter, bottom-up fashion also called multiscale modeling (21, 49). Here, we have conversely developed a simple phenomenological pharmacokinetic/pharmacodynamic model to describe the action of a single-drug metronomic regimen on tumor growth. This model was adapted from a previously published one (14) to describe gemcitabine pharmacokinetic/pharmacodynamic relationships in mice. Interestingly, this new model was able to fit experimental pharmacokinetic/pharmacodynamic data published more than 20 years ago by an independent group, thus demonstrating the potential of this type of top-down approach. After calibrating the model, simulations suggested that switching to metronomic scheduling could achieve higher efficacy over conventional regimen. Most interestingly, our experimental in vivo data confirmed the accuracy of the predictions, as 100 mg/kg weekly over 4 consecutive weeks showed no efficacy while 1 and 0.5 mg/kg daily significantly reduced tumor growth, as predicted by our mathematical model. Furthermore, simulations predicted that 0.2 and 0.1 mg/kg daily would be ineffective, thus highlighting the existence of a dose threshold. This is in agreement with the recent suggestion made by Bocci and Kerbel that metronomic chemotherapy might be better defined as a frequent, regular administration of drug doses designed to maintain a low, but active, range of concentrations of chemotherapeutic drugs during prolonged periods of time without inducing excessive toxicities (20). Of note, our simulations illustrate how PD effects are sensitive to changes in pharmacokinetic parameters. Indeed, when using different clearance values, the model was not able to accurately predict pharmacodynamic efficacy any longer. This observation highlights how dosing and scheduling must be closely adapted to individual pharmacokinetic parameters. Evaluation of interindividual variability thus appears critical prior to transplanting this approach in patients to drive metronomic dosing.

Because of the small size of the initial xenograft (i.e., <100,000 cells) and slow growing nature of the GI-ME-N neuroblastoma cells, no disease-related deaths were observed in any of the groups. Therefore, survival could not be compared between treatment groups. However, experimental data confirmed that both metronomic dosing regimens led to a 40%–49% reduction in tumor growth at the end of treatment as compared with untreated mice, while standard gemcitabine failed to induce any significant antitumor effect. In addition, our experimental data suggest that metronomic gemcitabine exerted potent antitumor effect as reduced vascular density and decreased tumor perfusion were observed in metronomically treated mice. This finding is consistent with previous studies demonstrating the antiangiogenic properties of metronomic gemcitabine (38, 39) but in contrast with other reports suggesting that metronomic gemcitabine may increase tumor perfusion and lead to vessel normalization (43, 50). This discrepancy may be due to differences in treatment regimens and/or time points at which tumor perfusion was assessed. Quite surprisingly, prolonged control of disease progression was observed in our study in metronomically treated mice (up to 2 months after treatment cessation) together with a 60%–72% reduction in tumor mass at study conclusion as compared with either control or standard gemcitabine. Although little evidence is available to pinpoint the underlying mechanisms, one can hypothesize that metronomic gemcitabine may impact on cancer stem cells (51) and/or stimulate the innate immune system nonabrogated in nude mice, thus explaining this sustained disease stabilization. Indeed, MC can lead to reinduction of tumor dormancy at least in part through immunostimulatory mechanisms (3), which has been proposed as a potential explanation for long-term disease control observed in some patients treated with MC.

Conclusion

Collectively, our results suggest that our pharmacokinetic/pharmacodynamic model could be a useful tool for in silico simulations to identify the best modalities for metronomic administration to be tested in priority in nonclinical models, thus avoiding testing regimens that are likely to fail in the clinic. This model has now been used with metronomic temozolomide in glioma (14), metronomic vinorelbine in lung cancer (23), and metronomic gemcitabine in neuroblastoma, but it could also be applied to other drugs commonly used in metronomic protocols, such as cyclophosphamide, capetabine, and etoposide, provided that extensive pharmacokinetic/pharmacodynamic data are generated and made available. Overall, our study indicates that phenomenological mathematical modeling could help guide optimal dosing and scheduling of chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: J. Ciccolini, D. Barbolosi, C. Meille, E. Pasquier, N. André

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