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USING COMPUTER SIMULATIONS FOR EVALUATING THE EFFICACY OF BREAST CANCER CHEMOTHERAPY PROTOCOLS

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The fundamental strategy of chemotherapy is to maximize tumor eradication within the limits of tolerable toxicity to the organism. To demonstrate the use of mathematical models in designing treatment protocols, we modeled the effect of chemotherapy on tumor mass and simulated the outcome of several neoadjuvant protocols for breast cancer disease. The model assumes unperturbed tumor growth, superimposed by periods of tumor regression during treatment applications. It takes into account both cell cycle specific (CCS) and cell-cycle non specific drugs (CCNS). Three possible modes of growth (exponential, Gompertz and power laws) were simulated in the study. The model parameters (such as cytotoxic activity of a given protocol) were estimated by best fit procedure from the clinical data of tumor regression following neoadjuvant treatments. The estimated parameters were then used to simulate various regimens that are employed today in the treatment of adjuvant and metastatic breast cancer. Our results suggest that although high dose chemotherapy (HDT) cannot eradicate overt metastatic disease, it may lead to cure if applied early in the natural history of breast cancer. Moreover, the simulations predict a better response for a rather toxic dose dense regimen, as compared to a more conventional protocol. However, our simulations suggest that a well tolerable continuous protocol is no less efficient. The results of the study provide insights into the effectivity of chemotherapy and may assist in designing better protocols.

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1. Introduction

Over the past several decades, there has been steady progress in the understanding of the pharmacokinetics and pharmacodynamics of cytotoxic agents.[5,10,19,36,48,52,61,69] In addition, the mathematical properties of cell growth of different chemotherapeutic regimens have been studied.[1,2,11,37,45,65] This research provides important insights as to the ways by which chemotherapeutic protocols can be improved for better tumor control. For example, it is demonstrated mathematically, and supported by *in vitro* and *in vivo* experiments, that the efficacy of CCS drugs will be increased if the frequency of their administration is modulated according to the frequencies of the cell divisions in the drug-susceptible host and cancer tissues.[3,12,67] Yet this quantitative knowledge is only rarely translated into the medical practice, and most of the chemotherapy protocols employed today are derived by a tedious, time and effort consuming, heuristic paradigms of clinical trials.[18]

The present work was aimed at theoretically examining the efficacy of several protocols which are currently employed in the chemotherapy of breast cancer. To do so we put forward a general model of chemotherapy in which we implemented three alternative modes of tumor growth: exponential,[59] Gompertz[34,44] and power law growth function,[14,40] which was recently shown to reflect the growth of primary breast cancer.[22] The model parameters (such as cytotoxic activity of a given protocol) were estimated by best fit procedure from the clinical data of tumor regression following neoadjuvant treatments.[9,55,57] We then simulated several clinical scenarios in the treatment of breast cancer, namely, treatment of primary tumor, of micrometastatic cancer, and of macrometastatic disease. The results of this study may assist in identifying among the many combinations of drug dosage and scheduling, the most promising protocols to be evaluated clinically.

2. The Basic Model

The mass specific growth rate of a tumor is the difference between the specific growth rate, g, at which its cells proliferate, and the specific rate at which they die per unit of tumor mass. The mass specific mortality rate is equal to the sum of the natural mortality rate m_0 and the mortality rate due to treatment, m_T. We assume that the specific growth and natural mortality rates g and m_0 are a function of the tumor size y. Moreover, we assumed that the specific treatment mortality rate depends on tumor size and on the treatment type and intensity. Hence the law of growth of an untreated tumor can be expressed in the form:

$$\frac{dy}{dt} = (g - m_0)y\,, \tag{2.1}$$

while the dynamics of treated tumor is described by:

$$\frac{dy}{dt} = (g - m_0 - m_T)y\,. \tag{2.2}$$

Let $f(y) = g - m_0$ be the net mass specific growth rate of the tumor when left untreated, so that (2.1) and (2.2) are now replaced by (2.3) and (2.4) respectively:

$$\frac{dy}{dt} = f(y)y\,, \tag{2.3}$$

$$\frac{dy}{dt} = [f(y) - m_T]y. \tag{2.4}$$

For exponential growth, the mass specific growth rate is a constant, $\gamma_{\exp} : f(y) = \gamma_{\exp} = \ln 2/t_{\exp}$, where $t_{\exp}$ is the tumor doubling time. For Gompertz growth, $f(y) = -\alpha \ln(y/y_{\max})$ where α is a constant and $y_{\max}$ is the limiting tumor size. For power law growth, $f(y) = \gamma_p y^{\beta-1}$ where γ_p and β are constants.

Both animal and cell culture experiments suggest that the mortality rate of cells due to chemotherapy (the dose response relationship) is often proportional to the drug concentration, c, and the mass of cells susceptible to the drug.[24,30,41,48,69] Cytotoxic drugs are usually divided into two classes: CCS drugs that are toxic to proliferating cells only, and CCNS drugs that are toxic to any living cell.[16,19,54] We assumed that the effect of CCS drugs is proportional to the mass of those cells that are actually proliferating. If we take as a reasonable approximation in actively growing tumors that g is proportional to the net mass-specific growth rate $f(y)$ then the effect of CCS drugs will be proportional to the net growth rate. On the other hand, the effect of CCNS drugs is simply proportional to the tumor mass y. Thus the mass specific mortality rate due to chemotherapy is:

$$m_T = r_s c_s(t) f(y) + r_n c_n(t)\,, \tag{2.5}$$

where c_n and c_s are measures of the concentrations of CCNS and CCS drugs at time t, respectively, and r_n and r_s are rate constants. The growth law during treatment is therefore:

$$\frac{dy}{dt} = \{f(y)[1 - r_s c_s(t)] - r_n c_n(t)\}y\,. \tag{2.6}$$

In practice, only the products, $k_s(t) = r_s c_s(t)$ and $k_n = r_n c_n(t)$ are needed for our simulations. Thus, the growth rate for treated tumor now becomes:

$$\frac{dy}{dt} = \{f(y)[1 - k_s(t)] - k_n(t)\}y\,. \tag{2.7}$$

3. Parameter Estimation

The parameters for the three possible growth laws for untreated tumors were estimated from the data of Fournier *et al.*[17] This study analyzes over 160 cases where tumor growth could be seen by consecutive retrospective mammograms. They reported an average tumor volume of 2.6 cc with a mean doubling time of seven months. This gives an estimate for the net growth rate for the exponential law of about $\gamma_{\exp} = 0.1$ month^{-1}. For the power law with $\beta = 0.5$, these data yield

an estimate of $\gamma_p = 0.135$.[22] In the case of Gompertz growth, using in addition a limiting size of $y_max = 3000$ cc,[46] the data give an estimate of $\alpha = 0.013$. The cell proliferation parameters for metastatic breast cancer were estimated from Spratt and Spratt,[58] using similar techniques (for the exponential law $\gamma_{\exp} = 0.25\ \text{month}^{-1}$; for the power law, $\beta = 0.5, \gamma_p = 0.22$; in the case of Gompertz growth, $\alpha = 0.03$).

We first estimated the functions k_s and k_n for neoadjuvant chemotherapy (primary breast cancer chemotherapy). This was then used to estimate the k_s and k_n of various protocols that are currently employed in the treatment of metastatic and adjuvant breast cancer. It was assumed, for the sake of simplicity, that there is a similar drug sensitivity for the cells of primary and metastatic cancer.

Chemotherapy protocols differ in the combination of drugs being used and the individual drug dosage and scheduling. To obtain a unified measure of drug efficacy between various drug protocols we extended a method developed by Simon *et al.*[32,56] Drugs in each category were converted to the dosage of a reference drug of the same category that gives a similar response. In the current study, the CCNS drug doses were all transformed to their equivalent doses of the drug doxorubicin; 1 mg of the doxorubicin was taken to be equivalent to 24 mg of cyclophosphamide, to 0.9 mg of thiotepa and 5 mg of carboplatin.[32] For the CCS drug category, 5-FU (5 fluorouracil) was used as a standard; 1 mg of 5 FU was taken as equivalent to 0.1 mg of methotrexate[32] and 0.1 mg of Taxol.[13] The various drug regimens that were simulated in this study are summarized in Table 1. The kill rate of combinations of drugs was calculated assuming that the drugs interact linearly.

We focused our analysis on the data of two studies[9,55] that provided the most detailed information of the primary tumor size changes during treatment. We used a less detailed study.[57] to corroborate the parameter estimates. The original studies report the tumor sizes before and following chemotherapy as assessed by clinical examination. A complete clinical disappearance of the tumor was categorized as a "complete response" (CR). If the tumor remained detectable after treatment, but its cross-section was reduced by more than 50%, it was classified as a "partial response" (PR). A response of less than 50% in the tumor cross-section was reported as "no clinical response"(NR). In order to quantitatively estimate the pathological tumor response to chemotherapy from this information, these data was coded as follows: NR = tumor volume remains at baseline; PR = 50% reduction in the cross-section of the tumor; CR = tumor size below the clinical detection threshold (taken as 0.8 cm; note that all these numbers are conservative). The accuracy of clinical size estimation was addressed by several authors[23,33] and generally a very good correlation was found between the clinical and the pathological size. Thus, the consecutive estimations of the average tumor volumes following treatment were taken as pathological size.

Numerous studies have shown that the most important factor governing the magnitude of the response (i.e. the proportion of the initial over the final tumor cross-section) of a drug during treatment is proportional to the total amount of

drug administered over that time. This is proportional to the integral:

$$\int_0^T c(t)\,dt\,, \tag{3.8}$$

where T is the total time of the treatment.[26,29,35,41] Thus, to a first approximation, a continuous constant drug dosage will have a similar effect to a sequence of pulses, provided that they have the same total dose. The assumption of constant dosage allows for considerable simplification in Eq. (2.7), as in most cases analytic solutions can easily be obtained. We therefore simulated solutions to Eq. (2.7) assuming that k_s and k_n were constant, and searched for values of these constants which gave the least squares fit to the data, using the Levenberg–Marquardt algorithm[39] as implimented in the *Mathematica* 3.0 computer package. Many different initial parameters were tested in order to guarantee that the global least squares fit had been obtained. From Eq. (2.6), the effect of a drug at any given time is proportional to concentration of the drug at that time. From this fact and the parameter estimates for constant dosage, the functions k_s and k_n could be estimated for pulse treatment. We checked that the fit to the data remained good when the assumption of a constant dosage was replaced with the actual pulse schedules (see for example Fig. 1).

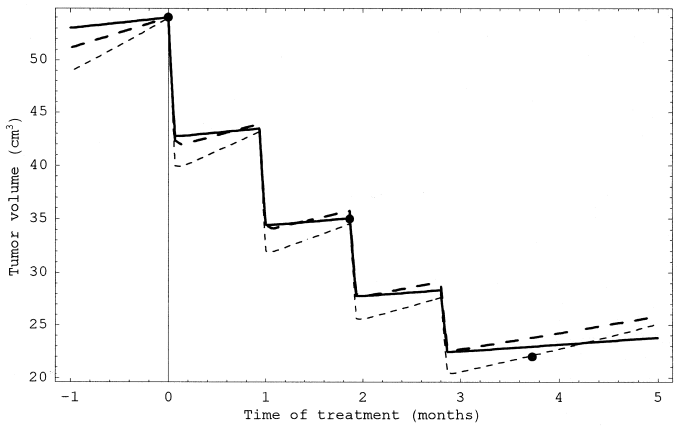


Fig. 1. Simulation of primary breast cancer response to pulse chemotherapy [Eq. (2.7)]. Parameters were chosen as the best fit to data.[17,55] The actual tumor volume is denoted by •. The simulation with power law growth is denoted by a heavy solid line, Gompertz growth by a heavy dashed line, and exponential growth by a light dashed line.

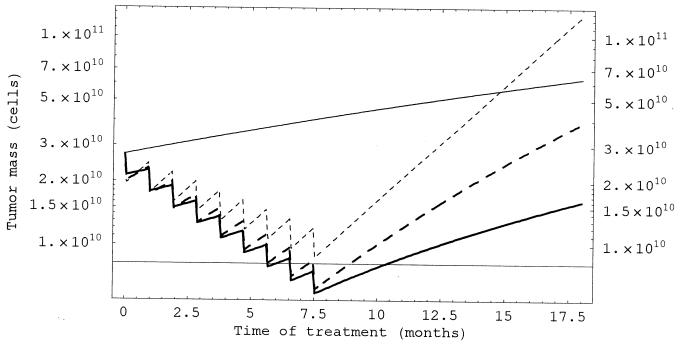
4. Chemotherapy Simulations

We used our model to analyze the effects of several currently employed treatments of metastatic and adjuvant breast cancer. Each treatment protocol was simulated in three clinical settings:

(a) an overt metastatic tumor burden of 10^{10} cells (tumor diameter of 3 cm);

(b) micrometastases which are below the clinical detection threshold of 10^7 cells (tumor diameter of 0.2 cm);
(c) very small micrometastases of 10^5 cells (tumor diameter of just 400 microns).

Figure 2 illustrates the simulation results of a conventional adriamycin based chemotherapy protocol CAF (cyclophosphamide, doxorubicin and 5-FU).[55] The results of the study provide insights into the effectivity of chemotherapy and may assist in designing better protocols. (The dosing schedules are listed in Table 2.) Figure 2a gives the effect of this treatment for metastatic disease. It is evident that the tumor burden is almost always incurable by such a regimen. This is true also for substantially smaller micrometastatic tumor burden (Fig. 2b). One should



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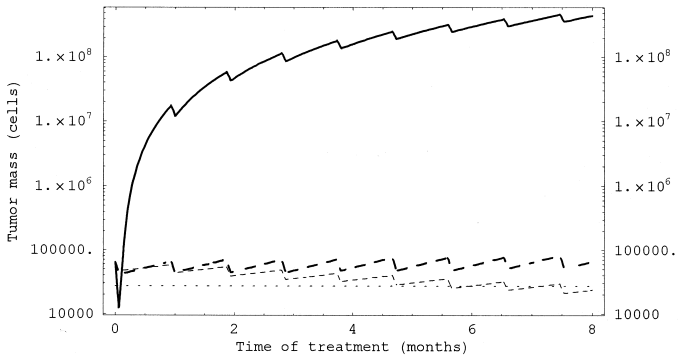


(b)

Fig. 2. Simulation of a conventional (CAF) pulsed chemotherapy of metastatic cancer. The treatment protocol and the cell kill parameters are given in Table 2. The y-axis represents the viable tumor cell number in log scale. The x-axis represents the duration of treatment in months. The three modes of growth are denoted as in Fig. 1. Untreated growth following the power law is denoted by a light solid line. (a) represents the treatment of overt metastatic disease (10^{10} cells). The clinical detection threshold of metastases is 10^9 cells; (b) illustrates a treatment of micrometastatic disease (10^5 cells). The dotted line represents the maximal possible size of an avascular tumor.

note, though, that breast cancer kinetic parameters have large variances. As a consequence, in very slow growing tumors, or in a very small metastatic disease, our conclusions may be too pessimistic.

It has been suggested that continuous long term infusion of cytotoxic drugs may provide better results than the conventional pulsed based protocols.[4,57] The simulation results of a continuous long term infusion are presented in Fig. 3. The simulations of neoadjuvant treatment are similar to those observed clinically[57] (Fig. 3a). The simulations of metastatic disease suggest that continuous long term infusion may give relatively good tumor response and even a chance of cure for small tumor burdens (Fig. 3c). However, note that a higher total dose was simulated in Fig. 3, as compared to Fig. 2, in order to mimic the clinical protocols of continuous drug administration (Table 2).

Our simulation technique was also used to evaluate the efficiency of high dose chemotherapy (HDT) in the adjuvant and metastatic setting (Fig. 4). Here we used doses of the drugs that were 3 to 15 times higher than those simulated in our conventional protocols, as is routine in intensive chemotherapy[6] (see Table 2). There is evidence that in such toxic dosages, the effect of many drugs cannot be characterized as only CCS or CCNS.[68] Thus, we assumed that the drugs simulated in Fig. 4 act through both mechanisms. From the model output, it is evident that HDT cannot cure large tumor burdens but is efficient in smaller tumors.

Gompertz (and power law) growth predict that the specific growth rate is higher in smaller tumors. This suggests that there will be an increased regrowth rate as the tumor shrinks. An approach based on this idea, assuming the Gompertz mode of growth,[45] is currently being evaluated in several clinical trials.[28] To combat regrowth, these studies simultaneously employ several intriguing approaches such as "late dose intensification" (increasing the dose level as therapy progresses), "dose dense" therapies (decreasing the rest periods between treatment pulses by using hematopoietic growth factors support), and "crossover intensification" (sequentially switching between relatively intense doses of different drugs). The strategy of these trials is simulated in Fig. 5 (see Table 2). Our results show that this intense treatment has a higher efficacy, but is nevertheless not capable of fully eliminating the tumor mass, except perhaps in very small tumors.

5. Discussion

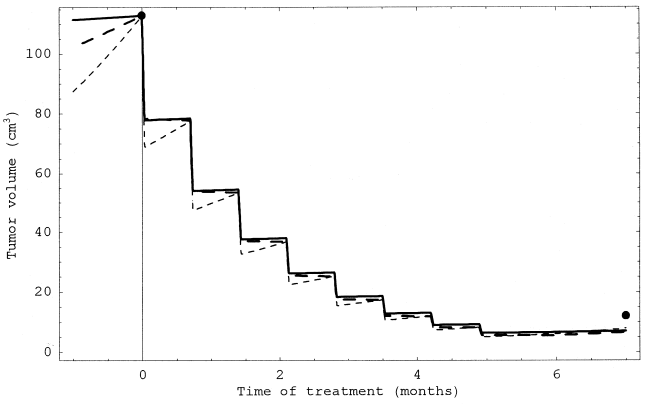
The mathematical model presented here provides a way to compare between different treatment strategies that are currently employed in breast cancer chemotherapy. The simulations predict that small metastatic tumors ($< 10^5$ cells) can be eliminated, but not overt metastatic tumors, which is in line with a recent meta-analysis of HDT clinical trials.[53] This work concludes that HDT does not improve the outcome of overt metastatic disease, but may be beneficial if applied early in the natural history of breast cancer. As expected, the simulations predict a better response for the rather toxic dose dense regimen,[28] as compared to the conventional (CAF)

Table 1. Breast cancer neoadjuvant chemotherapy data and estimated cell kill parameters. TED denotes total equivalent dose of a standard drug. All the doses are given as mg/m^2. P denotes the time period between consecutive courses. CTX stands for cyclophosphamide. MTX stands for methotrexate. The original protocol in Ref. 9 contained in addition vincristin 1 mg/m^2/21 d. It was since postulated that this drug did not contribute to efficacy of the protocol. The values of k_n and k_s parameters are given for three modes of growth simulated in the model. The parameters for the simulation in Fig. 3a were calculated using the mean k_n, k_s values as estimated from literature.[9,55]

Chemotheraphy protocols											Cell kill parameters					
											Power law		Exponent		Gompertz	
Ref.	# patients	CCNS drugs	Dosage mg/m^2/d	Days of treatment	TED dox.	CCS drugs	Dosage mg/m^2/d	Days of treatment	TED 5-Fu	P (months)	k_n	k_s	k_n	k_s	k_n	k_s
9	126	doxocubicin (dox.)	30	1	114	5 FU	400	4	2857	0.7	1.1	0.5	1.8	0.5	1.1	2.6
		CTX	300	4		MTX	20	2								
55	153	doxorubicin	25	2	98	5 FU	500	4	2143	0.9	3.5	0.9	4.5	1.3	3.5	3.5
		CTX	500	2												
57	50	epirubicin	50	1	169					0.7	11.2	0.3	16	0.3	11.2	1.1
		cisplatin	100	1		5 FU	200	30	6000	1						

Table 2. Breast cancer adjuvant and metastatic chemotherapy simulations: Chemotherapy protocols and corresponding model parameters. Notations are similar to that used in Table 1. For the high dose protocol, it is assumed that all the drugs used here act through both CCS and CCNS mechanism. In the dose dense protocol the drugs are use sequentially: Doxorubicin$*3 \rightarrow$ paclitaxel (taxol)$*3 \rightarrow$ cyclophosphamide$*3$.

Chemotheraphy protocols												Cell kill parameters: Power law		Exponent		Gomperts	
Ref.	Protocol	CCNS drugs	Dosage mg/m^2/d	Days of treatment	TED dox.	CCS drugs	Dosage mg/m^2/d	Days of treatment	TED 5-FU	P (months)	# courses	k_n	k_s	k_n	k_s	k_n	k_s
55	Conventional	doxocubicin (dox.)	50	1	131	5 FU	500	4	2857	0.7	9	8.7	1.3	12	1.7	8.7	5.7
		CTX	1000	1													
57	Continuous	epirubicin	50	1	169					0.7	6	11.2		15.6		11.2	
		cisplatin	100	1		5 FU	200	30	6000	1	6		0.3		0.3		1.1
6	High dose	CTX	1500	4	966	CTX	1500	4	25748	1	1	11.2	8.3	15.5	10.4	11.2	35.6
		Thiotepa	125	4		Thiotepa	125	4									
		Carboplatin	200	4		Carboplatin	200	4									
28	Dose dense	doxorubicin	90	1	180	paclitaxel	250	1	3111	0.5	3	16.7	8.1	23.2	10	16.7	34.4
		CTX	3000	1	250					0.5	3	23.2		32.2	10	23.2	



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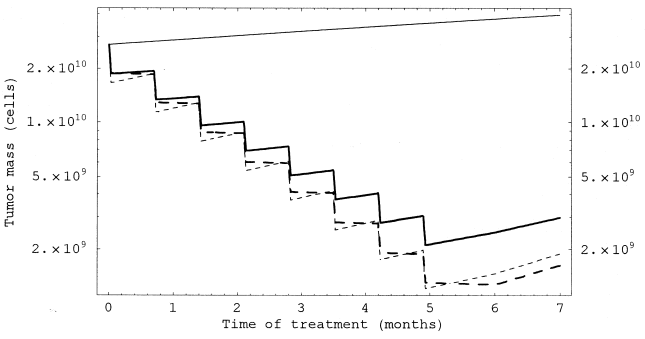

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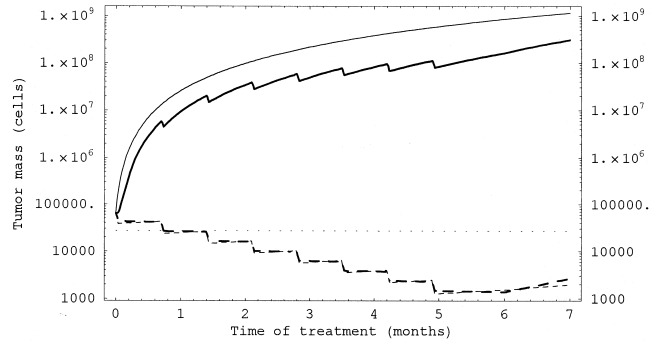
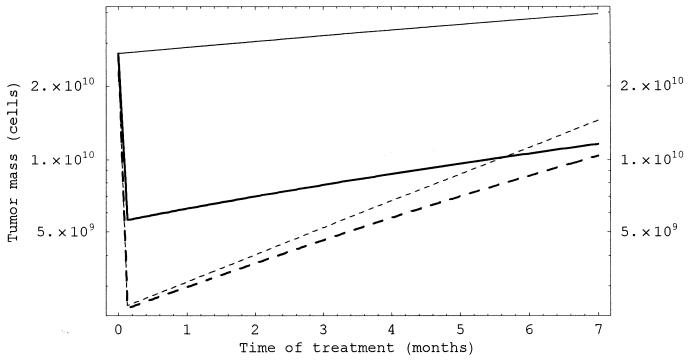


(c)

Fig. 3. Simulation of a prolonged continuous 5-FU treatment combined with pulsed chemotherapy for primary and metastatic cancer. The treatment protocol and the cell kill parameters are given in Table 2. The graph settings are similar to Fig. 2. (a) illustrates a neoadjuvant chemotherapy of primary breast cancer (growth parameters and notations as in Fig. 1). The actual tumor sizes drawn from a clinical trial using the same drug combination,[57] is given for comparison; (b) represents the treatment of overt metastatic disease (10^{10} cells); (c) illustrates a treatment of micrometastatic disease (10^5 cells).



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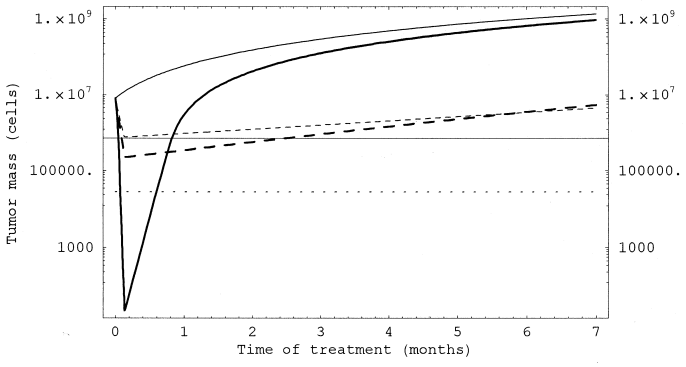

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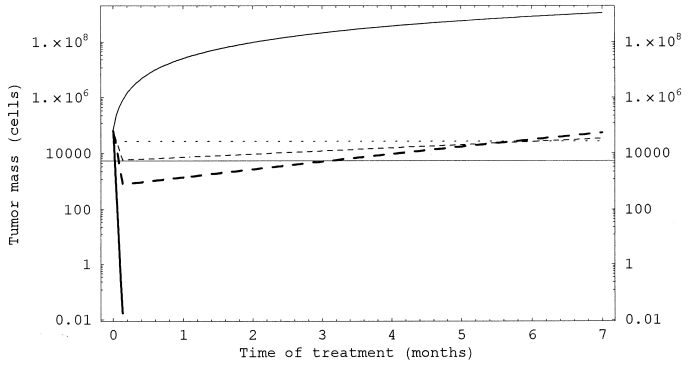
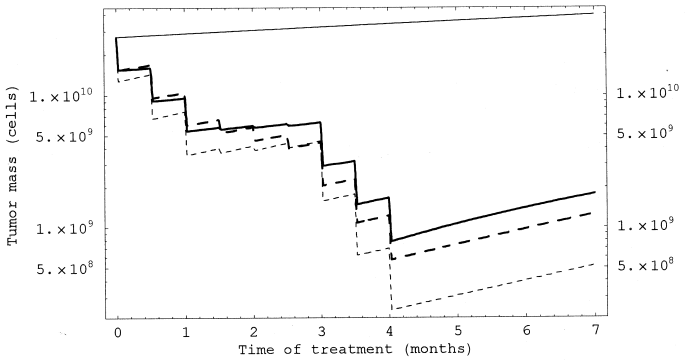


(c)

Fig. 4. Simulation of HDT for metastatic disease. The treatment protocol and the cell kill parameters are given in Table 2 (see Fig. 2 for notations). (a) represents the treatment of overt metastatic disease; (b) illustrates a treatment of a substantial metastatic disease (10^7 cells), yet below the threshold of clinical detection; (c) illustrates a treatment of micrometastatic disease (10^5 cells).



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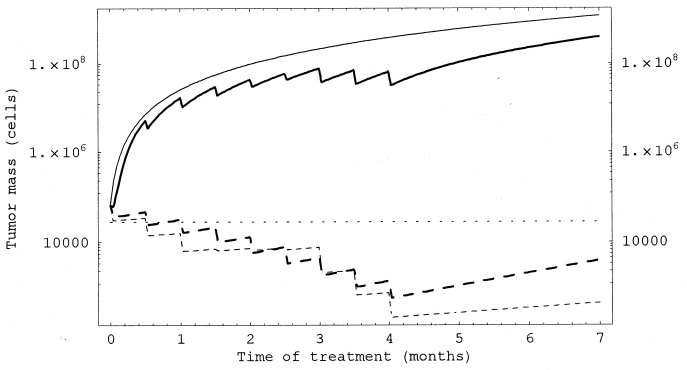

(b)

Fig. 5. Simulation of a pulsed dose dense chemotherapy for metastatic cancer. The treatment protocol and the cell kill parameters are given in Table 2. The graph settings are similar to Fig. 2. (a) represents the treatment of overt metastatic disease; (b) illustrates a treatment of micrometastatic disease.

protocol. However, our simulations suggest that the well tolerable continuous 5FU protocol[57] is no less efficient (see Figs. 3 and 5). Moreover, it is evident from our results that at least under the conditions simulated in this study, only HDT gives some hope of cure.

The exact tumor growth function may influence the results of different chemotherapy protocols. We have simulated three possible modes of growth for each chemotherapy protocol. Our results show that different modes of growth correspond to different responses to chemotherapy. For large tumors ($> 10^{10}$ cells), all three modes of growth predict qualitatively similar response to chemotherapy. The exponential growth predicted the poorest results since this mode of growth has the highest net growth rate in this size range, and therefore the fastest regrowth after chemotherapy (see Fig. 2a). In smaller tumor sizes ($< 10^7$ cells), power and

Gompertz law predict rapid regrowth of the tumor. Yet, the power law predicts a much larger specific growth rate as the tumor shrinks (especially $< 10^5$ cells). As a consequence, cells in very small tumors may be very sensitive to CCS drugs. This implies a more efficient activity of these drugs in small tumors. In such a case HDT may even eliminate relatively large metastatic burden of 10^7 cells. This result should be regarded with some caution, as for very small tumors, the power law gives an unrealistically large mass specific growth rate of $f(y) > 30$ month^{-1}, for tumor radius of 150μ (tumors with less than 10^5 cells). Note that this radius corresponds to the largest distance to a feeding capillary that is compatible with cell growth.[62,64] It should also be borne in mind that the mode of growth of avascular tumor may be different from that of large tumors, whose growth may be governed by the rate of angiogenesis.[25,63] In the current model, in the case of power law growth this phenomenon was accounted for by constraining the value of $f(y)$ from above by 15 month^{-1}.

In our model, we assumed that the effect of any given drug on metastatic tumors is the same as on primary tumors. This may not be the case in advanced disease, where a high probability of drug-resistance mutations exists.[15,21] However, the extent and the relevance of this phenomenon for breast cancer is not clear.[47] Our model does not allow for drug resistance *per se*. Nevertheless, it is evident from the simulations that the basic nature of Gompertz[45] and the power law growth[22] may provide an alternative explanation for the failure of chemotherapy protocols. As the treated tumor shrinks in mass, its specific growth rate increases, with a concomitant increase in the proliferation rate. The increasing growth rate of the shrinking tumor will eventually counterbalance the effect of chemotherapy, with a rapid regrowth as soon as treatment stops.[50]

The pharmacodynamical properties of chemotherapy modeled in the current study are relatively simple. For example, a simplifying assumption of an immediate reaction of cells to chemotherapy is implicit in the model. This is probably not a serious limitation because studies show that cells are eliminated as fast as one hour after chemotherapy application.[5,30,36] Thus, it may be assumed that the cell kill is instantaneous relative to the period of drug application simulated in the model (e.g., 24–48 hours). This duration of the pulse was chosen to correspond with the known pharmacokinetics of the drugs simulated in our study. Most of the chemotherapeutic agents that are simulated here have a half-life between 12 and 48 hours, and their pharmacokinetics may often be approximated by first-order kinetic rate equations.[41] The same method may be used to evaluate the efficacy of additional cytotoxic agents in new protocol design. In doing so one should remember that the classification of cytotoxic agents into CCS/CCNS, although useful, may be an over-simplification.

There is a great inter-patient variability in the cellular parameters[7,49] and in the response to cytotoxic agents.[43,51] It should be emphasized that the current analysis simulates only an average response of an average tumor. Treatment designs for individual patient may therefore require patient-specific information of

biological parameters such as Ki-67, TLI (thymidine labelling index) and S-phase fraction.[51,66,70,71] However, even though the simulations were based on rather crude and limited data, the model output was similar to what is observed in the clinics. Dedicated studies, using novel techniques, such as MRI and PET scan, for primary breast cancer,[8,20,60] and tumor markers and radioactive tracers for metastatic disease,[31,38,42] are required in order to provide more accurate estimates of tumor growth and response to chemotherapy.

Acknowledgments

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