*Mathematical Oncology*

# Adaptive Therapy

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## Abstract

**A number of successful systemic therapies are available for treatment of disseminated cancers. However, tumor response is often transient, and therapy frequently fails due to emergence of resistant populations. The latter reflects the temporal and spatial heterogeneity of the tumor microenvironment as well as the evolutionary capacity of cancer phenotypes to adapt to therapeutic perturbations. Although cancers are highly dynamic systems, cancer therapy is typically administered according to a fixed, linear protocol. Here we examine an adaptive therapeutic approach that evolves in response to the temporal and spatial variability of tumor microenvironment and cellular phenotype as well as therapy-induced perturbations. Initial mathematical models find that when resistant phenotypes arise in the untreated tumor, they are typically present in small numbers because they are less fit than the sensitive population. This reflects the "cost" of phenotypic resistance such as additional substrate and energy used to up-regulate xenobiotic metabolism, and therefore not available for proliferation, or the growth inhibitory nature of environments (i.e., ischemia or hypoxia) that confer resistance on phenotypically sensitive cells. Thus, in the Darwinian environment of a cancer, the fitter chemosensitive cells will ordinarily proliferate at the expense of the less fit chemoresistant cells. The models show that, if resistant populations are present before administration of therapy, treatments designed to kill maximum numbers of cancer cells remove this inhibitory effect and actually promote more rapid growth of the resistant populations. We present an alternative approach in which treatment is continuously modulated to achieve a fixed tumor population. The goal of adaptive therapy is to enforce a stable tumor burden by permitting a significant population of chemosensitive cells to survive so that they, in turn, suppress proliferation of the less fit but chemoresistant subpopulations. Computer simulations show that this strategy can result in prolonged survival that is substantially greater than that of high dose density or metronomic therapies. The feasibility of adaptive therapy is supported by *in vivo* experiments. [Cancer Res 2009;69(11):4894–903]**

### Major Findings

**We present mathematical analysis of the evolutionary dynamics of tumor populations with and without therapy. Analytic solutions and numerical simulations show that, with pretreatment, therapy-resistant cancer subpopulations are present due to phenotypic or microenvironmental factors; maximum dose density chemotherapy hastens rapid expansion of resistant populations. The models predict that host survival can be maximized if "treatment-for-cure strategy" is replaced by "treatment-for-stability." Specifically, the models predict that an optimal treatment strategy will modulate therapy to maintain a stable population of chemosensitive cells that can, in turn, suppress the growth of resistant populations under normal tumor conditions (i.e., when therapy-induced toxicity is absent). *In vivo* experiments using OVCAR xenografts treated with carboplatin show that adaptive therapy is feasible and, in this system, can produce long-term survival.**

## Background, Significance, Question

Systemic administration of cytotoxic drugs is the primary treatment strategy for patients with disseminated cancer. Whereas a wide range of treatment regimens are used in clinical practice, their fundamental goal is typically to induce lethal toxicity in the largest possible number of tumor cells (1, 2). Thus, most research efforts in chemotherapy are focused on discovery of agents and combinations of agents, doses, and dose schedules that maximally kill tumor cells while minimizing the toxicity to the host. In most clinical therapies, patient tolerance is the primary factor that limits the dose of cytotoxic agents. That is, cancer patients are usually treated at or near the maximum tolerated dose (MTD) with implicit intent to eradicate (cure) the tumor even when such an outcome, based on extensive clinical experience, is highly improbable.

Whereas many effective therapies are available, the amplitude and durability of tumor response to chemotherapy is limited by tumor cell resistance. This can arise due to either the intrinsic properties of the tumor cells or factors in the microenvironment (3, 4).

**Intrinsic factors.** (*a*) Phenotypic drug resistance. This may be through MDR1/ABCB1–mediated multidrug resistance, increased expression of p-glycoprotein, and increased DNA repair, as well as a number of other mechanisms (3–8). (*b*) Tumor cytokinetics. Most chemotherapy drugs are cell cycle specific so that tumors with low fractions of populations undergoing mitosis will be relatively resistant (9). (*c*) Evolution. Because cancer populations are typically heterogeneous and cancer cells are genetically unstable (resulting

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doi:10.1158/0008-5472.CAN-08-3658

## Mathematical Model Quick Guide

**Assumptions and equation building.** Our modeling approach (27) to therapy resistance in cancer begins with a tumor growth law (28). The only assumption that yields this result is that cancer cells, as a result of accumulating mutations, asymptotically approach a minimum information state necessary to maintain proliferation. This is manifested clinically as a progressive loss of differentiated function ("dedifferentiation").

**Equation 1:** $p(t) = Ct^{\Phi}C = (\Phi + 1)T^{-(\Phi+1)}$.

This is a probability density function $p(t)$ for the observation of a cancer during some period of time ($t$, $t + dt$). $C$ is a normalizing constant and $\Phi$ = 1.618... (the Fibonacci constant); $T$ is the total clinical observation time of the growth. We refer the mathematically inclined reader to the original article (27) for the derivation of this growth law. The clinically relevant prediction is that cancers in "free fields" (i.e., in the absence of applied therapy) will exhibit power law growth with an exponent of $\Phi$ (the Fibonacci constant, with value ~1.62). The free-field assumption is critical because it means that this growth rate should be visualized only in tumors that have no external constraints, such as chemotherapy.

If Eq. 1 describes growth in the absence of treatment, what growth law describes growth in its presence? Using methods developed in catastrophe theory (28), we have shown that the growth law is a modified version of Eq. 1:

**Equation 2:** $\underset{t\geq t_0}{p(t)} = C(t/t_0)^{\Phi}\cos^2[a(t)\ln(t/t_0)]$

Here $t_0$ is the time at which a therapeutic program of activity $a(t)$ is initiated. This may be thought of as imposing some time-dependent, external growth constraint on the tumor. In general, the term $a(t)$ represents the *effect* of therapy. Thus, in any given drug regimen, the values of $a(t)$ will vary in different tumor populations, depending on their environment and phenotype. Note also that this term is time dependent. Thus, it can change depending on nonheritable factors such as tumor perfusion or inducible intracellular factors that confer resistance.

The *cosine* term on the right side of the equation permits the addition of highly dynamic interactions. Note that in the absence of therapy [$a(t) = 0$], Eq. 2 returns to the same form as Eq. 1. On the other hand, effective therapy will, over time, tend to reduce the $\cos^2[a(t)\ln t/t_0]$ term toward 0 (28). The problem is, of course, that the tumor population is simultaneously attempting to grow according to the power law factor $C(t/t_0)^{\Phi}$ in Eq. 2. However, even therapy with a small degree of cytotoxicity, if it remains effective for sufficiently periods of time, can drive the tumor populations toward extinction despite the fast growth of the power law factor.

For our analysis of therapeutic strategies, we will define $p(t) = 1$ as the tumor burden that will induce death of the individual (~1 kg). The optimal clinical goal is to cure the cancer so that the therapy drives $p(t) \rightarrow 0$. However, an acceptable outcome is a therapy that maintains $p(t) < 1$. That is, a feasible therapeutic strategy is one that maintains a total tumor burden that is less than the fatal level of 1.

A complication is that, as the tumor population increases before therapy, it will inevitably become heterogeneous (i.e., develop *subpopulations* due to random mutations and/or microenvironmental changes). When therapy is applied to a heterogeneous tumor, each subpopulation $i$ will experience a different activity effect $a_i(t)$, so that the growth in the total tumor will be described as

**Equation 3:** $\underset{t\geq t_0}{p(t)} = \sum_{i=1}^{n} p_i(t)$

or, by Eq. 2,

**Equation 4:** $\underset{t\geq t_0}{p(t)} = \sum_{i=1}^{n} C_i(t/t_0)^{\gamma i\Phi}\cos^2[a_i(t)\ln(t/t_0)]$.

Statistically, these summations follow because the subpopulations act disjointly. That is, $p(t)$ describes the occurrence of the event "cancerous cell," and this occurs disjointly as either of tumor type 1 or of type 2, etc. In addition, the single normalization constant $C$ in Eq. 2 is now replaced by a generally different constant, $C_i$, for each subpopulation $i$, where it now represents the relative occurrence of subpopulation $i$ in the heterogeneous population.

Similarly, $\gamma_i$ represents a fractional decrease of the growth rate $\Phi$ in Eq. 2 due to the fitness of subpopulation $i$ relative to all others. This is determined both by phenotypic properties, which affect the growth rate, and by microenvironmental factors such as hypoxia that extrinsically reduce proliferation. Cells from very fit populations will have high $\gamma_i$ values, indicating quicker growth than other less fit components (i.e., those that are more sensitive to the total environment including chemotherapy). Conversely, for populations with low $\gamma_i$ values, it is postulated, as a summary effect, that either

in a high evolutionary rate), cytotoxic chemotherapeutic agents act as environmental selection forces that favor phenotypes that adapt to the chemotherapy by evolving resistant "strategies" such as those described above. Whether these resistant clones are present in small numbers in the original tumor or arise due to new phenotypes that evolve following commencement of therapy, the adaptive landscape of treated cancers favors regrowth of tumor due to proliferation of the resistant clones (10).

**Extrinsic factors.** (*a*) Poor perfusion. This limits drug delivery and potentially limits the effectiveness of cytotoxic drugs if the environment induces quiescence rather than proliferation (11). (*b*) Increased intratumoral interstitial pressure, which reduces drug flow into the tumor (12). (*c*) Hypoxia. Many chemotherapeutic agents require intermediate oxygen radicals of their cytotoxic effects (13). (*d*) Extracellular acidosis, which increases the activity of p-glycoprotein (14, 15). (*e*) Protective effects of tumor-associated mesenchymal cells.

Thus, drug resistance arises as a result of temporal and spatial heterogeneity in cancers that typically contain both multiple subpopulations and multiple microenvironmental subregions. Perturbation of a cancer by therapy sets in motion a number of interactions that not only result in tumor cell death but also promote phenotypic adaptation and alterations of microenvironmental conditions that may lead to evolution of resistance and regrowth. As is typical of nonlinear systems, these dynamics are very difficult to predict or control. By contrast, cancer therapy is typically imposed in a rigid fashion, with drug type, doses, and intervals fixed by protocol and altered only in the event of excessive patient toxicity. Thus, although the tumor is a dynamic system that evolves during treatment, therapeutic strategies tend to remain relatively static.

**Equation 5:** $\sum_{n=1}^{i} \gamma_n = 1 \text{ or } \sum_{n=1}^{i} \gamma_n \geq 1,$

depending, respectively, on whether there is a free-field environment (no therapy) present, or if, instead, some constraint on cellular proliferation is present, such as imposed therapy. However, these postulates have not yet been verified.

The form of Eq. 4 indicates that if therapy produces a value of $a_i(t)$ sufficiently large to overcome the growth term $(t/t_0)^{\gamma i \Phi}$, the tumor population $i$ will approach 0 with time. By comparison, if the values of $a_i(t)$ are uniformly 0 for all populations, Eq. 4 indicates that the tumor will exhibit a sum of power law growth terms, until some other constraint emerges (e.g., insufficient angiogenesis). We anticipate, however, that most therapies will have an intermediate degree of phase overlap with the tumor due to the presence some subpopulations with at least some phenotypic or microenvironmental resistance to therapy (Fig. 1), permitting regrowth. Furthermore, the tumor phase angle $a_i(t)\ln(t/t_0)$ in Eq. 4 can change with time. For example, this would occur if random mutations produce a resistant clone. Or, it can change due to a changing microenvironment that either increases or decreases the effectiveness of the therapy [i.e., the amplitude of $a(t)$]. Such changes can be induced by changes in drug concentrations (due to blood flow) or by environmental modulating factors such as hypoxia.

In this way, departures from the free-field assumption can be theoretically accommodated by suitably modeling the response of the tumor to chemotherapy. That is, any iatrogenic perturbation of the tumor represents a disruption of the free field, which can then be accommodated by the model.

In the next section, we will present a formal analysis of this model. To better visualize these results, we have also performed computer simulations that allow the system dynamics and model predictions to be graphically presented. The details of these simulations are presented below but essentially restate the modeling assumptions presented above.

## Findings

**Analytic results.** Prior analysis of the above model has been performed using catastrophe theory (27). This is now discussed from the viewpoint of the heterogeneous population growth effects.

In Eqs. 1-4, we model the effects of therapy on phenotypes with varying levels of sensitivity through their relative phases. Cells that are in phase with therapy are regarded as sensitive, whereas those that are out of phase have partial or complete resistance depending on phase difference. If all cells in the tumor were phase synchronized, then some therapy could eventually be discovered that would cure the cancer by killing all of the cells present. However, we propose that in a growing tumor with accumulating mutations and varying environmental selection forces, cancer cell populations inevitably undergo dephasing, so that resistant phenotypes emerge. In other words, finding a "magic bullet" that will cure a heterogeneous cancer consisting of multiple phenotypic and microenvironmental subpopulations is highly improbable.

The effects of this variability on therapy can be readily shown. Assume that the total cancer population is heterogeneous, consisting of subpopulations identified by different free-field fitness values. Call those with a given fitness value a "phase cohort." Just before initiation of therapy $a$, those cells with the maximum free-field fitness—the principal phase cohort—dominate the cancer mass. When therapy is initiated, it eliminates all cells that are in the principal phase cohort. Although a favorable outcome, it also sets the stage for its own failure. As shown in Fig. 1, the out-of-phase cells (those in the less-fit phase groups) may now start to grow freely because ($a$) they are unaffected by the therapy, and ($b$) they do not have the previously dominant (but therapy-sensitive population) to compete with them.

We then searched for an optimal, therapeutic strategy using a time-varying therapy schedule $a(t)$. We begin by defining, for convenience, a relative time variable $x = t/t_0$. Consider the activity schedule:

**Equation 6:** $\underset{1 \leq x \leq X_0}{a(x)} = (\pi/2)\min[a_0, (\ln(x + \Delta x))^{-1}], x \equiv t/t_0, \Delta x = \text{constant}$

Here $X_0$ denotes the relative time duration of the therapy and min denotes the smaller of the two terms in brackets. The min is taken to avoid an activity level $a$ that will harm the patient due to toxicity. Let quantity $\Delta x = \Delta t/t_0$ define the maximum uncertainty in the relative time due to a necessarily finite gap $\Delta_t$ between observations of the patient. The resulting mass curve $p(x)$ is, by substituting Eq. 6 into Eq. 2,

**Equation 7:** $\underset{1 \leq x \leq X_0}{p(x)} = Ax^{\Phi}\cos^2\left[(\pi/2)\frac{\ln x}{\ln(x + \Delta x)}\right] \text{ or } Ax^{\Phi}\cos^2[(\pi/2)a_0 \ln x]$

From Eq. 7, it is evident that the tumor burden $p(x) \to 0$ (i.e., a full elimination of the tumor burden) as the relative time $x \to \infty$. How fast is this approached? Of course, in this limit $\Delta x/x \to 0$. Therefore, we may expand out the $x$ dependence of Eq. 7 in a power series of $\Delta x/x$. This shows that asymptotically $p(x) \to 0$ as the function

A number of mathematical approaches have been developed to optimize chemotherapy and limit development of resistance. Probably the most well known and influential of these is the Norton-Simon model (16, 17), which found the treatment with the highest possible dose over the shortest period of time (maximum dose density). An important assumption in the Norton-Simon model is that tumor therapies fail because of evolution of resistant clones after therapy is started. High dose density is designed to produce maximal tumor cell death and minimize the potential for evolution of resistant clones. Thus, each patient typically receives chemotherapeutic doses at or near the limit of tolerable (or even fatal) side effects. In general, high-density chemotherapy has improved survival, but cure is achieved only rarely in most common epithelial tumors (18–22).

Recently, metronomic therapy has been proposed as an alternative strategy to the high dose density paradigm (23). This approach administers lower doses of drugs continuously or at frequent intervals without long interruptions. The fundamental rationales are ($a$) reduction of drug-induced toxicity and ($b$) increased treatment efficacy through a continuous antiangiogenic effect of conventional cytotoxic drugs (24). This represents an innovative strategy that implicitly abandons the focus on evolution of phenotypic drug resistance in the Norton-Simon model. However, it remains fundamentally attached to the concept that the optimal chemotherapy strategy (whether delivered over short or long terms) should use fixed protocols of drugs, doses, and timing and aim to kill the maximum possible number of tumor cells.

Here we use both mathematical models and *in vivo* experiments to examine the hypothesis that optimal therapeutic strategies evolve and change ("adaptive therapy") in response to intratumoral dynamics

**Equation 8:** $$p(x) \approx \lim_{x \to \infty} \frac{A\pi^2}{4} x^{\Phi} \left( \frac{\Delta x}{x \ln x} \right)^2 \sim x^{\Phi - 2} = x^{-0.382}.$$

The latter ignores the slower ln $x$ term. Hence, the best possible therapeutic outcome will result in a very slow monotonic decline of the tumor, but one that will never reach 0. Biologically, Eq. 8 shows that a durable stabilization of tumor burden can be achieved using a time-dependent therapeutic strategy because the fittest population is never brought to a complete extinction. In other words, a small population of the dominant, chemosensitive cell population is maintained, and these cells, in turn, suppress the growth of the chemoresistant clones that are less fit under free-field conditions. Although a complete cure is never obtained with this strategy, it does transform the disease to one that is a chronic but well-controlled process.

### Computer Simulations

**Tumor growth and effects of therapy.** The above modeling approach and results (27) provided the motivation for further evaluation of the dynamics of tumor cell proliferation during therapy and, in particular, the role of phenotypic (i.e., permanent) and environmental (i.e., reversible) resistance *in situ*. To accomplish this, we simplify the above general case to include only two or three subpopulations and explicitly include microenvironmental factors in both tumor growth and response to therapy.

The presence of environmentally-induced drug resistance due to hypoxia and insufficient blood flow is assumed to be dependent on tumor size (29, 30). Conversely, stabilization of tumor volume may result in maturation (normalization) of intratumoral blood vessels, increasing flow and oxygen concentrations (31). The goal of this component of the work is to use computer simulations to test high dose density, metronomic, and adaptive therapies in tumors with various combinations of chemoresistant (both phenotypic and microenvironmental) and chemosensitive populations.

**Computer simulations.** For free growth, as outlined in Eq. 1, we assume each subpopulation $i$ within the tumor will grow according to the iterative expression

**Equation 9:** $$P_i(t+1) = P_i(t) \times (1 + [\gamma_i \times G]).$$

The term $\gamma_i$ represents the replication rate of each subpopulation $I$, and $G$ represents the competition for resources (nutrients, space, growth factors, etc.) among different populations. In addition,

**Equation 10:** $$G = \frac{\gamma_T \times \Sigma P_j}{\Sigma(\gamma_j \times P_j)}$$

The term $\gamma_T$ is the maximum theoretical replication rate of the entire tumor. During therapy, the population will proliferate linearly with $\gamma_i$ and $G$, but decline linearly according to a "death" function $d(t)$,

**Equation 11:** $$P_i(t+1) = P_i(t) \times (1 + [\gamma_i \times G]) \times (1 - d(t)), d(t) = a(t)\beta(t)\sigma_i$$

where $a(t)$ is the therapy dose (or intratumoral concentration), $\sigma_i$ is the phenotypic sensitivity of the population $i$ to the therapy, and $\beta$ is the environmental sensitivity. In the simulations, we assume that $a \geq 0$, $0 \leq \sigma \leq 1$, and $1 \leq \beta \leq 2$.

Thus, if the therapy is administered at $t = t_0$, the tumor growth dynamics will include combining the proliferation and death terms as separate factors,

**Equation 12:** $$P_i(t_0 + 1) = P_i(t_0) \times (1 + [\gamma_i \times G])[1 - a(t_0)] \times \beta(t_0) \times \sigma_i.$$

### Environmental Sensitivity

The presence of environmentally-induced drug resistance due to hypoxia and insufficient blood flow is assumed to be dependent on tumor size (29, 30). Conversely, stabilization of tumor volume may result in maturation (normalization) of intratumoral blood vessels, increasing flow and oxygen concentrations (31).

In our model, the environmental sensitivity is $\beta$, which is constrained to a value between 1 and 2 and will increase with time as long as the tumor size remains stable,

**Equation 13:** $$\beta(t) = 1 + \frac{t}{t + \tau_S}.$$

By the form of Eq. 5, $\tau_S$ is the time required for the environmental sensitivity to increase from 1 to 1.5 in a tumor with stable size. To accommodate the assumed change in environmental sensitivity with tumor growth, we assume that $\beta$ increases with time if tumor size remains stable, and decreases with time as the tumor grows. If tumor volume Vol($t$) increases, the new environmental sensitivity will be $\beta' = \alpha\beta$, where $\alpha = \text{Min}\left(\frac{\text{Vol}(t-1)}{\text{Vol}(t)}, 1\right)$. In this case, the function that defines the environmental sensitivity is shifted in time by a factor $\delta(t)$ as depicted below:

**Equation 14:** $$\beta(t) = 1 + \frac{t - \delta(t)}{t - \delta(t) + \tau_S}; \delta(t) = \frac{(\alpha - 1) \times (t + \tau_S) \times t}{(\alpha - 1) \times (t - \tau_S)}.$$

that included therapy-induced perturbations in the environment and evolution of resistance strategies. We address the question: Can adaptive therapeutic strategies improve survival compared with high dose density or metronomic therapies when tumor heterogeneity (25, 26) produces phenotypically and environmentally induced resistant tumor populations before initiation of therapy?

## Meaning and Implications

**Summary of modeling results.** In the modeling section, we have used two different approaches. The first is an analytic approach that generally examines the tumor cell sensitivity to cancer therapy by assigning a phase to both the cell and the therapy. If the phases of the cell and treatment completely overlap, the cell is killed; if they are completely out of phase, the cell survives; and if there is some overlap, a varying probability of survival can be assigned. The latter allows for intermediate levels of resistance. Note that the phase of the tumor cell and chemotherapy can be changed by environmental factors (i.e., hypoxia or ischemia) as well as, for example, the phenotypic resistance of the tumor cell. As a cancer cell population increases, it is reasonable to assume that accumulation of random mutations and heterogeneous blood flow will cause significant "dephasing" of the cells so that variable levels of sensitivity to any therapy will likely be present. This is a key point in the model: If resistant populations are present before administration of therapy, traditional therapeutic strategies that seek to kill as many sensitive cells as possible actually promote the rapid growth of resistant cells. The reason is that chemosensitive cells are ordinarily fitter than resistant phenotypes because the latter must expend additional substrate and energy to carry out the resistance strategy. In the absence of therapy, the

### Modeling Adaptive Therapy

Ideally, adaptive therapy will vary both the drug and dose density depending on the status of the tumor. In these simulations, we will examine only dose density by modifying the amount of drug given. The algorithm for dose density adjustment used in the model is (see Fig. 2)

$$\text{Equation 15:} \quad a_{i+1} = \text{Min}\left(a_i \frac{\text{Vol}(i+1)}{\text{Vol}(i)}, \text{MTD}\right)$$

Here, $a_i$ and $a_{i+1}$ are the dose density administered previously and the new density, respectively; Vol($i$) and Vol($i$+1) are the volumes of the tumor before and at the time of dose administration, respectively; and MTD is the maximum tolerated dose.

### Simulations

To extend some of the analytic results, we simulated various treatment strategies in tumors that contain subpopulations that possess different fitness values as well as varying sensitivity to chemotherapy due to phenotypic or environmental resistance.

Five different population phenotypes were considered in the simulations:

($a$) FS, fittest population in free-field growth but completely sensitive to therapy.
($b$) S, less fit in free-field conditions and sensitive to therapy.
($c$) R, less fit in free-field conditions and resistant to therapy.
($d$) FR, fittest population in free-field conditions and phenotypically resistant to therapy.
($e$) ER, phenotypically sensitive to therapy but in an environment that confers resistance.

These populations were combined in four different scenarios as depicted in Fig. 3. To each combination, we applied the following therapeutic strategies: MTD, adaptive therapy, metronomic therapy using continuous drug perfusion or intermittent, fixed doses at high or low frequency, as described in Table 1. The outcome of therapy was measured by the tumor size and life expectancy (i.e., the time for tumor to reach a total burden that was judged to result in death). These values can be seen in Fig. 3. Typical growth dynamics for different combinations of populations and therapeutic strategies are shown in Fig. 4.

**Maximum tolerated dose.** The results for treatment with MTD show that the strategy of killing as many tumor cells as possible in the beginning of treatment leads to a considerable reduction in tumor size if the dominant (fittest) population is also sensitive (i.e., the FS population). However, after this populations has been substantially reduced or eliminated, the resistant populations begin to exhibit increased growth. In the case of the environmentally resistant cells, this is because the reduction in tumor growth normalizes their ischemic hypoxic environment. If phenotypically resistant but less fit populations (R) are present, they are able to increase their proliferation rate because the fitter populations (FS) has been eliminated by therapy and is no longer a competitor.

Note that if the fittest population is also resistant (FR), no therapeutic strategy was effective.

In all of the simulations (except for the FR population), the MTD strategy yielded the lowest life expectancy.

**Adaptive therapy.** Adaptive therapy initially delivered the worst results because the tumor does not decrease in size as much as with the other approaches. However, as time progresses, maintaining a stable small population of the FS population does reduce the growth of the resistant phenotype (R), resulting in only very slow tumor growth and prolonged patient survival. When environmentally resistant populations are present, the environmental sensitivity increases and the resistant population decreases if the tumor volume is kept stable. This is consistent with the concept of vascular normalization as a mechanism to enhance therapeutic effects. In this scenario, the patient will survive indefinitely. In addition, note in Fig. 3 that in this scenario, at the end of adaptive treatment, all of the extant tumor cells are sensitive, indicating that high-dose therapy with curative intent would be possible in this tumor. Finally, the simulation consistently showed that, when using adaptive therapy, tumor control could be achieved using chemotherapy doses that decreased with time.

**Metronomic therapy.** The principle of metronomic therapy is to apply chemotherapy doses that are less intense but more frequent than the standard MTD protocol (including continuous infusion). We find that this approach does consistently yield better results than MTD. The consistent administration of lower doses results in slow reduction of the sensitive population (FS). This stabilization of tumor increases the sensitivity of the environmentally resistant populations (ER), which enhances therapy. Similarly, because some members of the FS population remain during much of the early phase of metronomic therapy, they are able to suppress the phenotypically resistant population (S). The negative point of metronomic therapy is that throughout the time of therapy administration (which is increased due to more frequent application), the overall fitness of the FS population will be reduced, thus limiting its ability to completely suppress the resistant phenotype (R). This results in thresholds at which the resistant populations break through and begin to grow faster, leading to tumor recurrence and growth to a fatal level.

sensitive population will proliferate at the expense of the resistant phenotypes. However, if therapy removes all of the sensitive cells, the resistant population can proliferate freely because it does not have to compete with any fitter population. In contrast, we find that if therapy maintains a fixed population of chemosensitive cells, they will, in turn, suppress the growth of resistant cells, allowing the overall tumor burden to remain stable.

Although likely be a concept difficult for physicians and patients to accept, the model suggests that treatment strategies, under these circumstances, should abandon "cure" as an implicit or explicit goal. Rather, treatment of disseminated cancers using therapies with no significant probability of cure should focus on controlling, but not eliminating, the sensitive cells so that they can, in turn, reduce the growth of the resistant populations.

In the second modeling approach, we use numerical simulations to better illustrate the findings in the analytic solutions and examine the outcomes in several possible treatment scenarios. This is particularly valuable in separating phenotypic (i.e., permanent) resistance from environmentally induced (i.e., transient and potentially reversible) resistance. This allowed explicit testing of the adaptive therapy model and comparison with more standard strategies. The results suggested that adaptive therapy, if it could be successfully applied, will permit longer survival than that obtained with high dose density or metronomic strategies. Based on these results, animal experiments were done to determine the feasibility of using adaptive therapy *in vivo*. Those results are described below.

## Experimental Studies

Experimental testing of some of the modeling results was

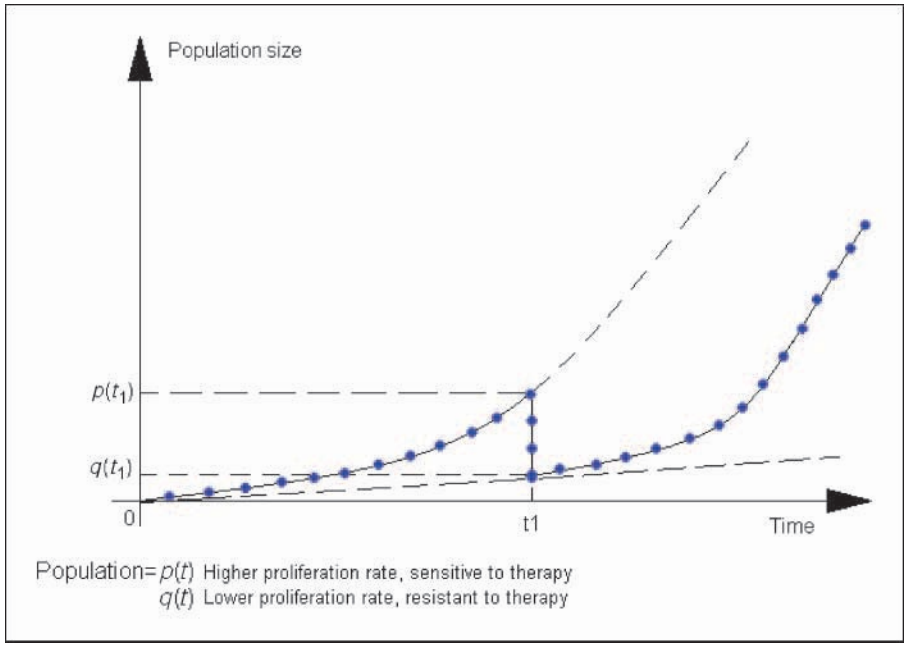



**Figure 1.** Simulation of treatment dynamics in a tumor with a dominant subpopulation ($p$) that is sensitive to some treatment and a small subpopulation ($q$) that is less fit (resulting in slower proliferation) but completely resistant to the treatment. Therapy is applied of time $t = t_1$ and is sufficient to entirely eradicate subpopulation $p$. The tumor size initially decreases. However, the resistant phenotype is now also the fittest extant population and rapidly regrows, resulting in tumor regrowth and resistance to any further therapy.

carried out in an ovarian cancer (OVCAR-3) tumor cell line growing in severe combined immunodeficient mice and treated with carboplatin administered i.p. Tumors were established by injecting a slurry of $10^6$ OVCAR cells into the s.c. tissues in the flank of female severe combined immunodeficient mice (4–6 weeks of age). When the tumors grew to a volume of $\sim 300$ mm$^3$, the mice were divided into three groups: control, a "standard therapy" arm (60 mg/kg i.p. q4 days $\times$ 3), and adaptive therapy. The adaptive group received an initial dose of 50 mg/kg and thereafter the tumors were evaluated every 3 days and the dose was adjusted to maintain a stable tumor volume. The algorithm for dosing basically represented "a shot in the dark" because no prior experience was available to parameterize the models. Drug doses were established in increments of 10 mg/kg starting at the starting dose of 50 mg/kg. A treatment decision was made at the time of each measurement. If the tumor remained stable (defined as the no more than a 10% change from the prior volume using caliper measurements), no drug would be administered. If the tumor diminished in size or remained stable for two or more measurements, the next dose would be decreased by one 10 mg/kg decrement. If the tumor increased in size greater than 10%, the same dose of drug would be administered. If the tumor again increased in size, the dose would be increased to the next higher level.

The experimental results are shown in Fig. 5 and represent the mean tumor burden for four animals in each group. The initial experiment confirmed that therapy could be modulated in a way that allows persistence of a small stable tumor. However, some obvious limitations were apparent because modest tumor growth in the adaptive therapy animals did occur. Based on this experience, we repeated the experiment to more rigorously maintain stable tumor volume.

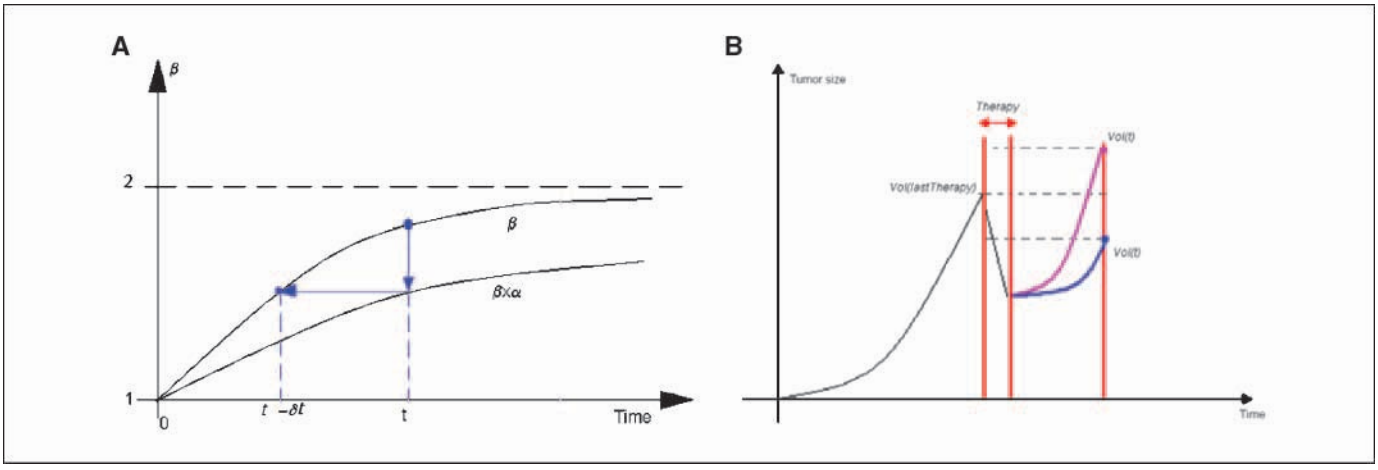
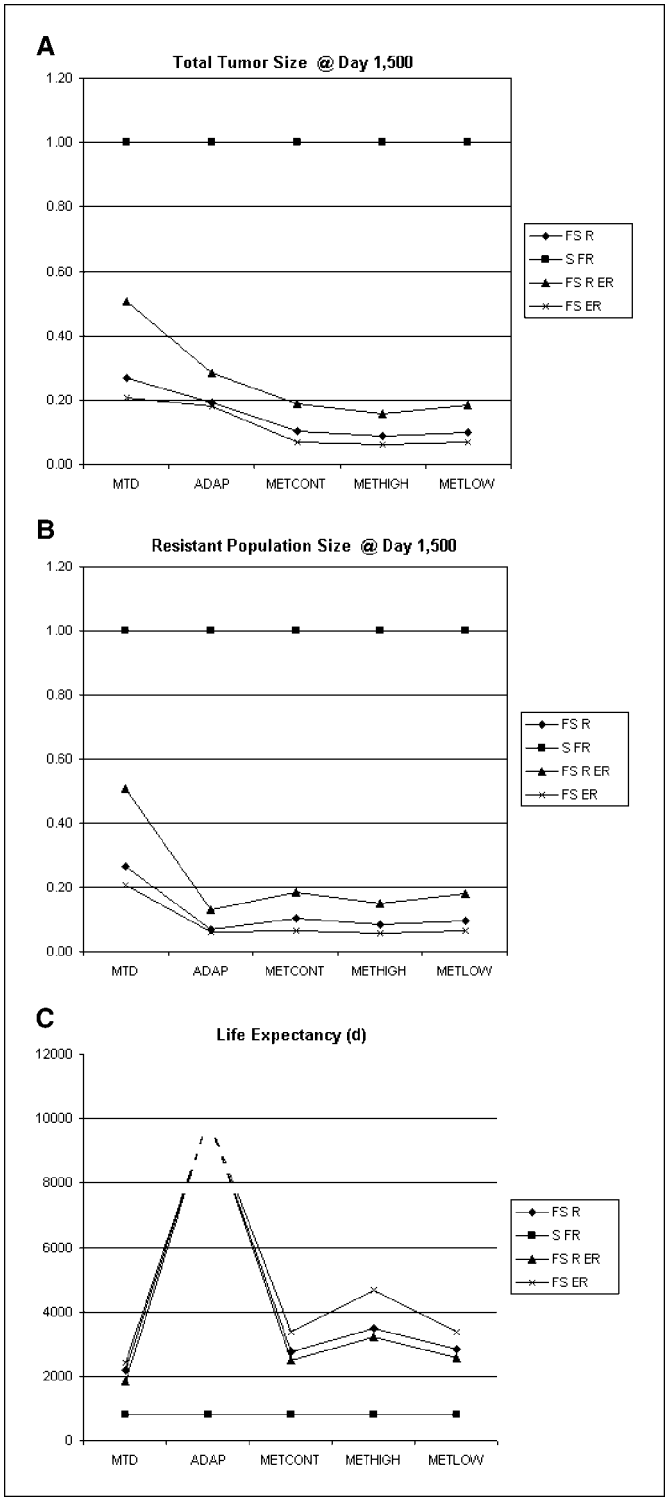



**Figure 2.** *A,* algorithm used for adjusting the environmental sensitivity. Once a tumor increases in size, the environmental sensitivity is decreased by the inverse of the growth rate. A delay of $\delta$ is then added to the original function so that the environmental sensitivity still grows asymptotically toward 2 but will require more time. *B,* dynamics of tumor regrowth and adaptive therapy treatment. Before the therapy is applied, the tumor is measured, and its size compared with the value at the prior time step. Should the tumor be bigger, the therapy intensity is increased; if the tumor is smaller, the dose density is decreased.

Results from this experiment are shown in Fig. 5 and continue to show that animals receiving adaptive therapy could survive indefinitely with a small, reasonably stable tumor burden (with both slow increases and slow decreases in tumor volumes observed). The total amount of carboplatin administered to the conventional group was 180 mg/kg, whereas the two adaptive groups received 320 and 310 mg/kg, respectively. The standard therapy induced a substantial response, but the tumor eventually recurred resulting in death. In both experiments, tumor volume stability could be enforced by a modulated treatment strategy. In both cases, the amount of drug necessary to maintain tumor stability decreased substantially with time so that at the completion of the experiment, control was achieved with administration of 10 mg/kg of carboplatin.




## Discussion of Mathematical and *In vivo* Results

Cancers are highly dynamic and adaptive systems that can evolve phenotypic strategies to overcome proliferation barriers in their environment. Application of therapy to the tumor adds an iatrogenic selection force to the adaptive landscape that will inevitably promote evolution of therapy-resistant phenotypic strategies. This evolutionary capacity to develop resistance results in treatment failure and tumor regrowth in the vast majority of cancer patients. Interestingly, despite this well-recognized dynamic and evolutionary nature of tumors, cancer chemotherapy is typically applied through protocols that *a priori* fix the drug(s), dose, and timing.

Virtually all current chemotherapy regimens have, as a fundamental strategy, the goal of killing maximal numbers of tumor cells. Usually, this is achieved through application of the highest drug dose that results in acceptable patient toxicity. More recently, metronomic therapy has been proposed as an alternative strategy. This approach uses smaller doses of drugs given in shorter, regular intervals or continuously to reduce toxicity and increase the antiangiogenic effects. This strategy maintains fixed dosing schedules and retains, as an explicit goal, effecting maximum tumor cell death.

Clearly, the ideal cancer therapy is one that identifies and successfully attacks the key fitness parameters in all extant tumor subpopulations as well as their adaptations to therapy, so that, despite the tumor heterogeneity and evolutionary capacity, complete eradication is obtained. Currently available therapies do seem to achieve this goal in some relatively homogeneous tumors such as testicular cancer and Hodgkin's lymphoma. Long-term response can be achieved in many other cancers and sarcomas, but resistance almost invariably emerges, ultimately resulting in tumor progression and patient death.

Here we explore a conceptual model of cancer treatment that we call adaptive therapy. A general principle of adaptive therapy is that cancer treatment should be as dynamic as the tumor populations that are being treated. Specifically, therapeutic strategies should evolve in response to and in anticipation of tumor adaptation through continuous adjustment of drugs, dose, and timing.

Using mathematical model and computer simulations, we find that treatment with the explicit or implicit intent to cure neither

**Figure 3.** Simulations for application of five therapy strategies: (*a*) MTD; (*b*) adaptive therapy; (*c*) metronomic therapy (continuous infusion); (*d*) metronomic therapy (high-frequency administration); and (*e*) metronomic therapy (low-frequency administration). Four combinations of mixed cell populations that include FR with high free-field fitness and high sensitivity to therapy, and R with lower fitness and low sensitivity to therapy, S with low fitness and high sensitivity, and ER with high intrinsic sensitivity and fitness but in an environment that restricts proliferation and response. Combinations shown in (*a*) "FS and R," (*b*) "S and FR," (*c*) "FS and R and ER," and (*d*) "FS and ER." The results show that at day 1,500 of tumor growth (1,100 d after initiation of therapy), the tumor treated using the MTD strategy was largest whereas those treated with metronomic therapy were smallest. When the simulations were run until the tumor burden achieved the lethal threshold, all patients in the MTD and metronomic therapies succumbed to their disease. However, the tumors treated with adaptive therapy remained stable even after a period exceeding 10,000 d.

**Table 1.** All therapies analyzed in a cycle of 60 days

| Therapy strategy | Dose density | Interval (d) | Total dose (60 d) |
|---|---|---|---|
| MTD | 50 | 60 | 50 |
| Adaptive | 25 (initial) | 60 | Variable |
| Metro continuous | 0.83 | 1 | 50 |
| Metro low freq. | 8.33 | 10 | 50 |
| Metro high freq. | 4.16 | 5 | 50 |

NOTE: The first strategy tested is MTD with one high dose per cycle. The second strategy used was the adaptive therapy with an initial dose intensity of 25 every cycle (half of MTD). Three different strategies were used for metronomic therapy; the doses were administered on a daily basis, once every 5 d and once every 10 d, respectively. In all cases, except for adaptive therapy, the total amount of therapy administered in a cycle of 60 d is the same.

completely eradicates all tumor cells nor even achieves a maximum length of survival if resistant populations are present at the time therapy is initiated. The dynamics that lead to this finding arise through the Darwinian interactions of cell subpopulations in which therapy-resistant cells are, in the absence of therapy, less fit than the sensitive populations due to the phenotypic cost of resistance. That is, the utilization of energy and other resources to, for example, increase xenobiotic metabolism reduces the amount available for proliferation.

Our analysis shows that, in the absence of therapy, the fitter, chemosensitive cells actually suppress the growth of the less fit but resistant population. Therapies designed to kill maximum numbers of cancer cells produce an environment in which the resistant cells both survive and are unopposed by the fitter, chemosensitive populations. This permits rapid regrowth of a therapy-resistant cancer. Alternatively, if therapy is limited to allow a significant number of chemosensitive cells to survive, they will, in turn, suppress the growth of the resistant population. We hypothesized that under these circumstances, adaptive therapy should be designed to maintain a normal cohort of surviving sensitive cells.

Preliminary testing of this theoretical modeling shows that adaptive therapy can achieve a substantially longer survival than standard high dose density strategies by maintaining a tumor volume that is either stable or slowly increasing for a prolonged period of time. We show that metronomic therapy will achieve a longer survival than standard drug regimens, but low-dose therapy applied continuously or at frequent intervals will still tend to

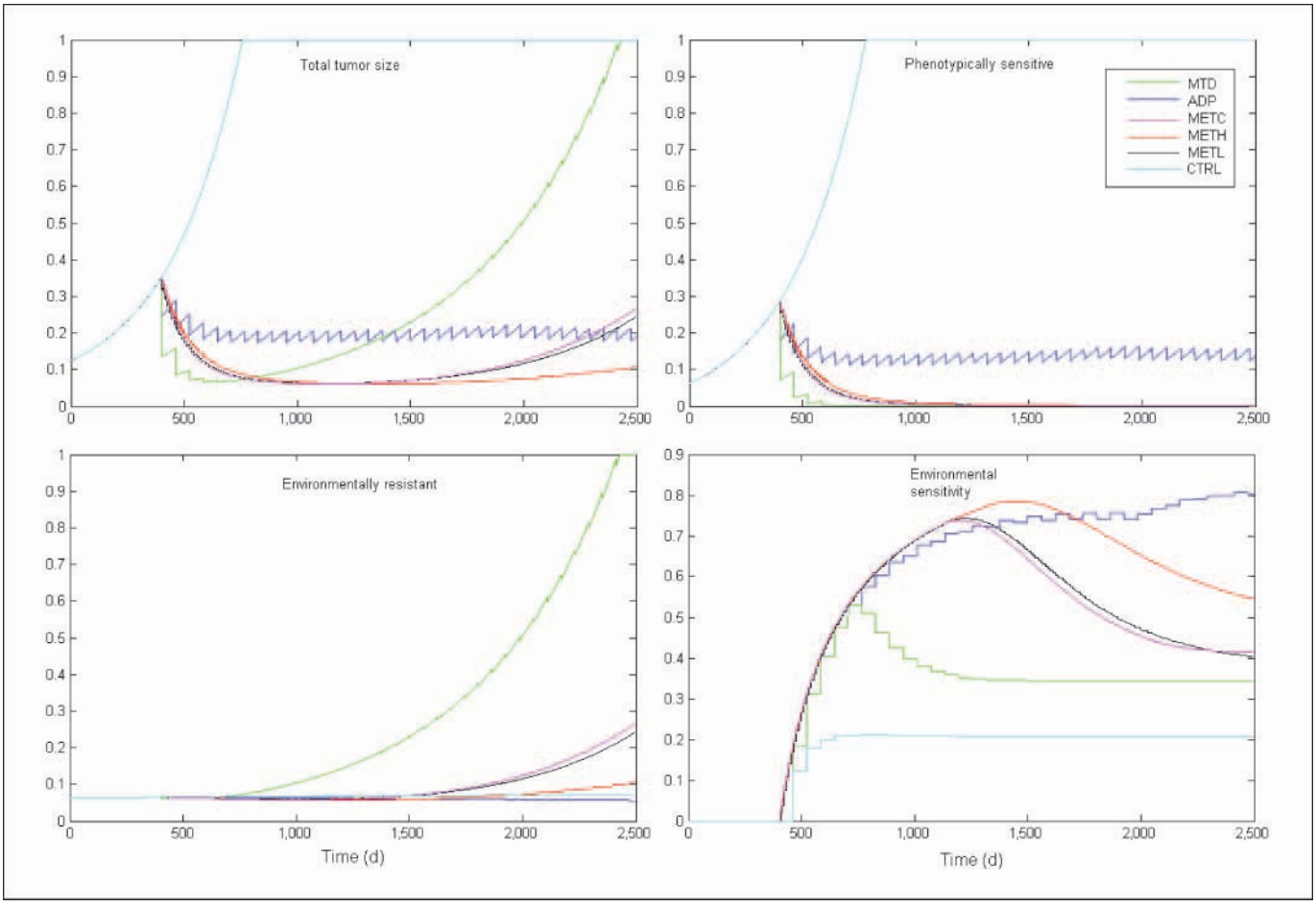



**Figure 4.** Progression of tumor properties with time in initial response to different therapeutic strategies. All graphs use two populations, FS and ER, the first being fit and therapy sensitive, the second environmentally resistant. The top left graph (*Total tumor size*) shows that MTD therapy results in a better initial result but the tumor promptly acquires resistance and recurs. Metronomic therapy (*METC, METH*, and *METL*) also reduces the tumor and is able to keep it stable for longer, but eventually, the resistant populations emerge once sensitive population is depleted (*top right*, *Phenotypically sensitive*). As shown in Fig. 3, these resistant populations result in tumor regrowth and patient death. Tumors treated with adaptive therapy (*ADP*) decrease in volume much less than with the other therapies but then maintain a stable tumor size for a prolonged period of time. Control (*CTRL*) corresponds to untreated tumors.

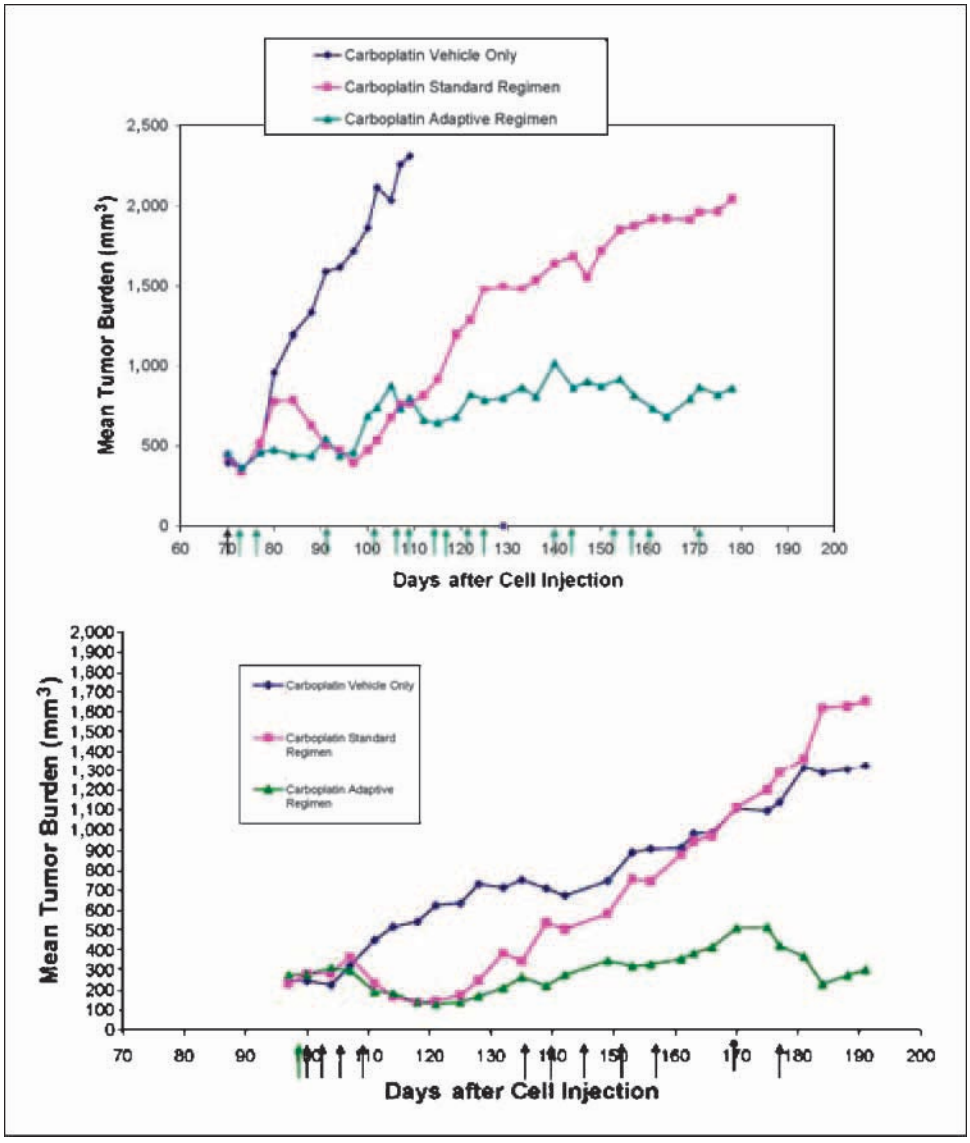



**Figure 5.** Two different experiments as described in the text. The *y*-axis is the mean tumor volume for the four animals in each experimental group, and the *x*-axis is the time from s.c. inoculation of $10^7$ tumor cells. Each experiment included four animals in three experimental arms: (*a*) control (vehicle only); (*b*) "standard" high dose therapy consisting of 60 mg/kg q4 days for 3 doses; (*c*) adaptive therapy which begins with a dose of 50 mg/kg and then adjusts the dose to maintain a stable tumor volume. The arrows on the *x*-axis represent days in which therapy was given in the adaptive group. In the top experiment, the doses are (from left to right) 50, 40, 40, 30, 30, 20, 20, 10, 10, 10, 10, 10, 10, 10, 10, 10 mg/kg. In the lower experiment, the doses are 50, 50, 40, 40, 30, 20, 20, 10, 10, 10, 10, 10, 10 mg/kg.

promote growth of resistant populations leading to tumor regrowth and patient death. We find that adaptive therapy can maintain a stable tumor population for a prolonged period of time, permitting long-term survival.

We present some experimental results that establish the feasibility of using adaptive therapy. To the best of our knowledge, no *in vivo* studies using this therapeutic approach have been previously attempted, and so our initial work was essentially exploratory to determine if stable size could be achieved in an aggressive tumor model using the principles of adaptive therapy. The experiments represented only a simplistic test of the model because the therapy variables were limited to the dose and timing of a single drug (a complete adaptive therapy would also include alternative drugs) and assessed tumor response only by changes in size. Nevertheless, our results do confirm that a prolonged stable tumor volume can be achieved through application of the principles of adaptive therapy.

Although limited, the experimental results raise additional interesting questions primarily because we found that control of tumor could be achieved using progressively lower doses and increasingly long intervals between doses. In the simulations for tumors in which the primary mechanism of resistance is microenvironmental, we found that enforcing a constant tumor volume allowed "normalization" of the intratumoral vasculature. This permitted tumor control with decreasing amount of drugs and, more importantly, resulted in an end point in which all of the tumor cells were sensitive to the chemotherapeutic agent. If this is confirmed experimentally, we note that this progression toward maximal sensitivity over time during tumor volume stabilization may offer an additional adaptive therapy strategy in which high dose density cytotoxic drugs could be administered with maximal effectiveness after an initial therapy aimed to maintain a constant size.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

Received 9/24/08; revised 2/10/09; accepted 2/12/09.

**Grant support:** NIH grants P20 CA113004-01 and R01 CA77575-05.

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