Evidence for Power Law Tumor Growth and Implications for Cancer Radiotherapy

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Abstract Understanding tumor growth dynamics is important for planning cancer therapy, especially in radiotherapy, where standard fractionation is determined based on the assumption that tumors grow exponentially. In this chapter, we explore the ramifications of an alternative, but equally plausible, assumption of tumor growth, namely power growth law, realized in linear increase in tumor diameter. We present a simple model for tumor growth, whose global dynamics demonstrate power law growth of the tumor, even under unlimited nutrient supply. For corroboration, we carry out and analyze one-, two- and three-dimensional tumor growth experiments both *in vitro*, in MCF-7 cells (breast cancer cell line) and *in vivo*, in mouse xenografts. In all studied cases, the unsaturated growth follows a power law growth function. Simulating radiotherapy under power law, Gompertz and exponential tumor growth, we show that the power law model predicts radically different outcomes for the conventionally used treatment. This demonstrates the importance of using the appropriate tumor growth model when calculating optimal dose fractionation schedule for radiotherapy.

1 Introduction

Tumor growth has been intensively studied mathematically, as its dynamics crucially determine the success of oncotherapy. In radiotherapy and chemotherapy, where treatment is often given in fractions and the tumor regrows in the time intervals between cycles, it is important to take tumor growth pattern into account when planning the scheduling of treatment. However, definitive understanding of macroscopic tumor growth dynamics is still lacking.

It is widely accepted that tumor growth may obey exponential, Gompertz or logistic growth laws (e.g., [11, 13]) and clinical treatments are usually performed according to standard schedules, which were set based on these assumptions. However, these assumptions have never been biologically proven. A recently published paper doubts the validity of Gompertz growth, which motivates the current policy of adjuvant chemotherapy, and raises serious doubts about its underlying experimental evidence [18]. Moreover, new experimental data imply constant growth of tumor diameter, i.e., power law tumor growth [2], as was also inferred from mathematical analysis of mammography screening trial data [8]. Although several mathematical models for chemotherapy schedule planning considered different growth laws (e.g., [16]), only few previous studies have suggested a power law tumor growth rate [3, 5, 8, 22].

In radiotherapy, one can analyze the dynamic effects of different radiation schedules using the relatively good clinical documentation and the relevant

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mathematical models. Available mathematical models of cancer radiotherapy have two parts: radiation effect module, for assessing tumor cell survival after a single dose of irradiation, and tumor regrowth module, which describes what happens to the tumor between successive radiation doses. Together, these models can be used to design optimum treatment protocols, with the goal of minimizing tumor size as well as minimizing complications to host tissues. Wheldon et al. [25] presented an analytic formula for an optimal schedule, using the linear quadratic (LQ) model for radiotherapy effect and assuming exponential tumor growth. More recent works discussed the effect of tumor repopulation, considering different growth laws, e.g., [13, 14, 19, 23]. These models are usually based on non-linear growth model of the tumor, with initially exponential behavior, progressively slowed down by various limiting factors, such as hypoxia. These models were used in developing several clinical treatment schedules which were implemented into the clinical practice [15]. One example is the standard fractionation schedule [21], consisting of 30 daily $2Gy$ irradiation fractions. Other schedules are used for various cancers according to the specific tissue response to radiation. For example, hypo-fractionation (a smaller number of larger-dose fractions) is used for treatment of prostate cancer [4], whereas accelerated fractionation (same total dose, but shorter overall treatment time) is applied for head and neck cancer [17].

In this chapter, we question the assumption of exponential or Gompertz tumor growth law. Our theoretical model, corroborated by experimental results, suggests that the conventional assumption that tumors grow exponentially during the unsaturated stage, is not necessarily valid, and that power law may be the function most descriptive of solid tumor growth. Model simulation results for radiotherapy treatment outcome demonstrate how different choices of tumor growth model may lead to dramatically different research conclusions.

The rest of this chapter is constructed as follows: in Sect. 2 we present a model for macroscopic tumor growth, based on a previous model for cancer cell dynamics, for which simulation results had shown power law tumor growth [22]. To test the predictions of the model, we have conducted experiments, measuring tumor proliferation dynamics in one, two and three dimensions. Experimental results support the power law tumor growth (Sect. 3; the computational and experimental methods are detailed in Sect. 6). Implications of power law growth on the clinical outcomes of radiation therapy are tested by integrating both our suggested tumor growth model and the exponential and Gompertz models into the LQ model for radiotherapy effect. It is shown that the predicted effects of the standard fractionation schedule radiotherapy on tumor size, are largely different if, indeed, the tumor obeys the power law growth model (Sect. 4). As summarized in Sect. 5, we conclude that the possibility of polynomial tumor growth law should not be dismissed, and that prospective trials are necessary for its verification. Our study shows that basing predictions of clinical therapy outcomes on a doubtful assump-

tion of tumor growth law may lead to erred conclusions about the preferred therapy schedules.

2 Power law model – linear growth of tumor diameter

In a previous work [22], tumor growth was explored using a cellular automata (CA) model, originally presented in [1] for describing developing tissues. Numerical simulations of this model were conducted to obtain the macroscopic dynamics of tumor growth. For the two-dimensional (2D) CA, we observed that the time course of total number of cells in the unsaturated stage of growth (before space/nutrients limitations are imposed) could be well approximated by a parabola, i.e., it is proportional to the square of time, suggesting power law tumor growth [22]. We also simulated a one-dimensional (1D) CA, which showed linear growth of the total number of cells. Thus, we observed that both in 1D and 2D cases, tumor diameter grows linearly in time.

The following global dynamics, observed in the simulation results of the CA model, now comprises the underlying assumptions of a new macroscopic model for the unsaturated stage of growth. It is important to note that these assumptions are in agreement with the experimental observations of spheroid growth [12]. The assumptions are as follows:

- the cell colony has a spherically symmetric form with diameter $D(t)$;
- for some constant ℓ, in the inner area of depth larger than ℓ (inner sphere of radius $0.5D(t) - \ell$), the cell population is homogeneous and in equilibrium (i.e., net growth rate is zero);
- in the rim between radius $0.5D(t) - \ell$ and $0.5D(t)$ the cell densities are dependent on the relative position in that rim but not on time; cells in this rim have a net growth rate which depends on depth (distance from boundary) only;
- the cell mass expands due to cell population growth, but the properties of the inner mass and the outer rim remain invariant.

These assumptions imply that the diameter of the cell mass grows at a constant rate, regardless of the dimension (to see that, note that a unit surface area produces a constant number of cells per time unit, thus increasing the outer radius by a constant length). Therefore, we obtain the following equation

$$\frac{dD(t)}{dt} = k,$$

where k is the constant rate of increase in diameter.

From our macroscopic model, it directly follows that the diameter of the occupied area will grow linearly in time during the unsaturated stage. In the 2D case we can write the following expression for the total cell number

$$n = \frac{\pi D^2 \rho}{4},$$

where ρ is cell density. Substituting $D(t)$ from above equation yields

$$n = \left(\frac{k\sqrt{\pi \rho} t}{2} + \sqrt{n_0} \right)^2, \tag{1}$$

where n_0 is the initial cell number. In the same way, cubic growth equation can be derived for the three-dimensional (3D) case.

The last equation represents parabolic cell population growth, as indeed appears in the simulations of the original CA model [22]. We subsequently calculated k for different combinations of parameter values as an estimation of the macroscopic growth rate. Importantly, the relative densities of different cell types in the inner part of the cell population during unsaturated growth phase were indeed identical to those found in the steady state phase, enabling calculation of the parameter ρ (data not shown). This proves that the assumptions of the macroscopic model presented here are coherent with simulation results of the cellular automata model in [22].

3 Comparison with experimental results

In order to validate our model, we conducted *in vitro* experiments, where breast cancer cells from MCF-7 line were seeded in thin channels and in Petri dishes, and their growth was monitored. We also carried out *in vivo* experiments and analyzed growth dynamics of breast cancer xenografts in mice. To see whether power law model can be more or less preferable than other models in describing these data, we compared goodness of fit to that of exponential model; note that in this case, of initial stage of growth, a Gompertz or logistic model will predict exponential behavior too.

3.1 In vitro experiments: 1D and 2D growth of cancer cell colonies

In the thin channels, where cell colonies could only grow in one dimension, the progressing front line of the colony, was imaged every 30 minutes for several days (see Sect. 6.1 for details). To draw the front line of the colony, we performed an image processing analysis using Matlab image analysis tools. The average position of the front line at each time step was displayed, showing linear growth in each of the nine different replications (Fig. 1). Next, we performed linear fit and calculated the front line progression rate from the

linear slopes (see Sect. 6.4 for computational methods). The average progression rate of the colonies' front lines was $16.2 \mu m/h$ ($SD = 2.07$).

Colony growth in two dimensions was measured in 15 MCF-7 colonies, seeded in Petri dishes and monitored daily. Microscope images were analyzed using GIMP software and the area occupied by the colony was defined. The area measurements, obtained for 15 colonies and a quadratic fit was performed for each of the colonies separately (for methods see Sect. 6.2, 6.4). Shown in Fig. 2 is an example of quadratic fit for several colonies. From the parameters of the quadratic fit, radius growth rate was calculated for each of the colonies, under the simplifying assumption of circlular structure of the colonies. The calculated average growth rate was $11.6 \mu m/h$ ($SD = 3.41$). It can be seen that the area of each colony closely follows parabolic growth, in agreement with our model (average $R^2 = 0.964$), implying linear radius growth. Comparison to the goodness of exponential fit ($R^2 = 0.990$) shows that the benefit for the exponential model is insignificant. Overall, these results suggest that power law model is plausible for 1D and 2D cancer cell colony growth.
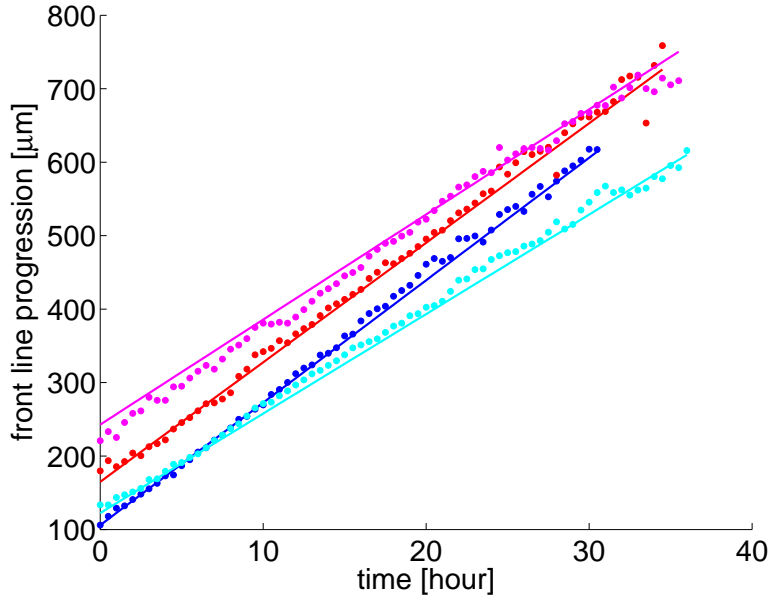

Fig. 1 1D front line progression of MCF-7 cell colonies shows a linear growth pattern. Shown are the results of four out of the nine colonies examined, each denoted by a different color. The colony growth in a thin channel was experimentally measured in 30 min intervals (dots). Slopes of the linear fit (lines) of all the different independent replicates suggest similar growth rates.

3.2 In vivo experiments: 3D growth of xenograft tumors

To check the validity of our model predictions *in vivo*, we carried out and analyzed growth dynamics of breast cancer xenografts (EMT-6 cell line) in mice (for methods see Sect. 6.3). In this experiment, tumor volume in the xenografts was monitored for a month or more, and cubic fit was performed for each of the tumors separately. Results for four representative experiments out of the nine conducted are shown in Fig. 3. Goodness of fit ($R^2 = 0.980$) was slightly better than for exponential model fit ($R^2 = 0.975$). Here too, radial growth rate was calculated for each experiment by using the cubic model fitted parameters, assuming spherical structure. The calculated average growth rate was $16.0 \mu m/h$ ($SD = 2.14$). The experimental measurements of tumor volume were used to calculate tumor radius in the same time points, assuming spherical structure. These data points are plotted in Fig. 4 along with the linear curves resulting from the fitted power law model. It can be concluded that *in vivo* tumor dynamics is also consistent with linear growth of the tumor diameter.
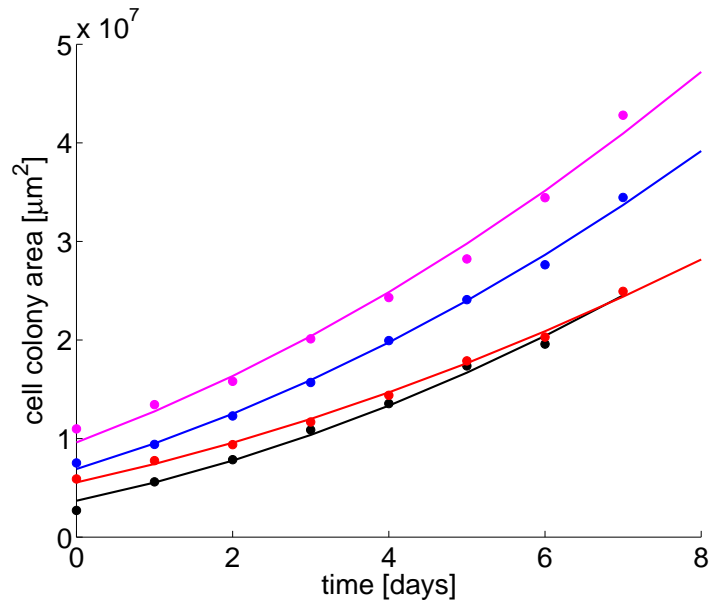

Fig. 2 2D progression of MCF-7 cell colonies show quadratic growth pattern. Shown are four out of the fifteen colonies examined, each denoted by a different color. The colony growth in Petri dishes was experimentally measured daily (dots). Parameters of the quadratic fit (lines) were used to calculate radial growth rate, resulting in similar values for all the different independent replicates.

4 Implications for radiotherapy

In order to examine the possible effects of a specific tumor growth law assumption on the choice of the radiation therapy schedule and on the clinical outcomes, we compare the power law growth model with the two most commonly assumed models of tumor growth, namely the exponential and the Gompertz (limited growth) models. To evaluate tumor response to each single irradiation dose, we use the LQ model. In 2007, McAneney and O'Rourke [13] examined the effect of tumor growth models on the results of specific radiotherapy schedules, using the LQ model to assess the radiation effect, and considering exponential, Gompertz and logistic growth models to evaluate tumor repopulation in the intervals between radiotherapy applications. They show that, under standard treatment protocols, exponential and logistic models yield similar results for tumor eradication, while for tumors described by Gompertz model calculations give poorer prognosis. In the spirit of [13], we integrate either exponential or Gompertz growth law models with the LQ radiotherapy effect model, and also examine our suggested power law model in the same method. In this way we can compare theoretically the effects of power law tumor growth on radiotherapy treatment results, to that of other models of tumor growth.
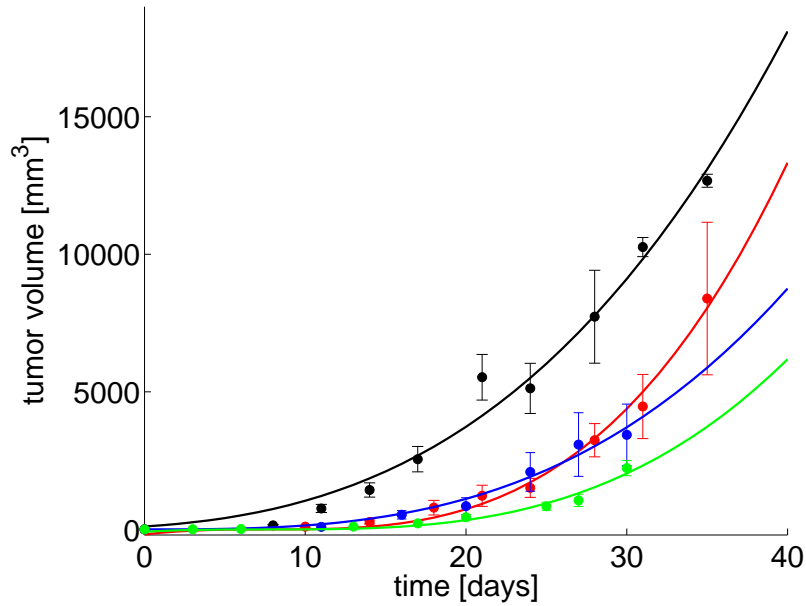

Fig. 3 Tumor volume measured in xenografted mice shows good fit to cubic growth model. Shown are four out of the nine experiments results (dots with SD bars), each denoted by a different color. Parameters of the cubic fit (lines) were used to calculate the constant radial growth rate.

4.1 Tumor growth models

First, we define the three different growth models that we consider. The exponential model is given by the equation

$$\dot{N} = rN, \tag{2}$$

where N is the tumor size and r is the constant growth rate. The solution of this equation yields

$$N(t) = N(0)\exp(rt), \tag{3}$$

where $N(0)$ is the initial tumor size at $t = 0$. The Gompertz equation for tumor growth is

$$\dot{N} = -gN\ln{(N/K)}, \tag{4}$$

where g is the growth rate parameter, and K is the tumor carrying capacity, i.e., the maximal size a tumor would reach without treatment. The solution for this equation is

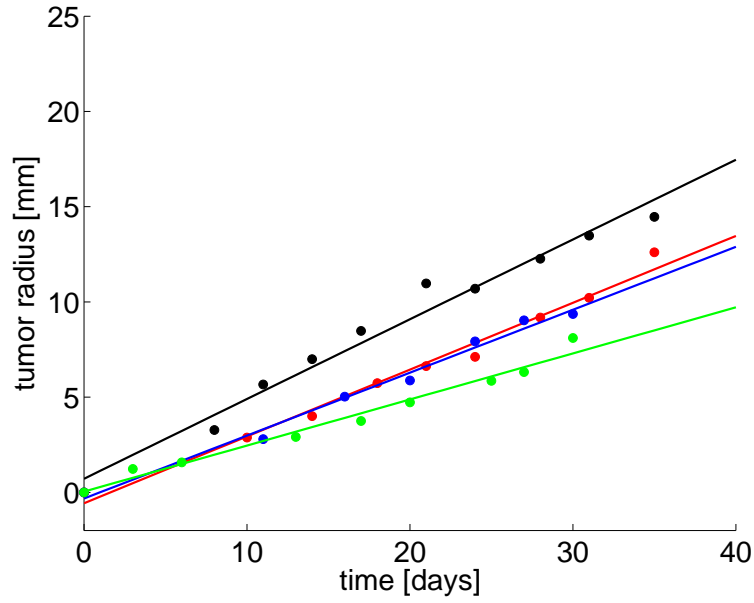$$N(t) = K\exp{[exp(-gt)\cdot\ln{(N(0)/K)}]}. \tag{5}$$

Fig. 4 Tumor radii in xenografts show linear growth. Shown are results for four out of the nine experiments, each denoted by a different color. Tumor radius (dots) was calculated from tumor volume measurements, assuming spherical structure of the tumors. The linear radius growth model (lines) with the fitted radial growth rate is shown for each experiment.

The power law model, resulting from our previously described assumptions, takes the following 3D form:

$$\dot{N} = 3AN^{\frac{2}{3}}, \tag{6}$$

where A is proportional to the linear growth parameter for the radius of the tumor. The solution for the latter equation is

$$N(t) = (At + N(0)^{\frac{1}{3}})^3. \tag{7}$$

4.2 Integrating tumor growth model with the LQ model describing radiotherapy effect

Next, we estimate the survival fraction (SF) of the tumor following radiotherapy, i.e., the ratio between the number of tumor cells at the end of treatment, and the number of tumor cells just prior to the first treatment (marked here as N_0). The values of SF are calculated for each tumor growth model, using LQ model for radiation effect. The treatment is assumed periodic, and is defined by its period, τ, number of applications, n, and dose per application, D. Here, we assume that the therapy is applied at times $t = 0, \tau, 2\tau, ..., (n-1)\tau$ and that the effect is immediate. We use the standard LQ expression for the effect of the radiotherapy per application: $\sigma = e^{-D(\alpha + \beta D)}$, where σ is the SF after irradiation, and α, β are the linear and quadratic coefficients in the standard radiotherapy effect model, describing cell survival after irradiation [10, 20]. The ratio α/β is a measure of the tissue sensitivity to radiation dose. Low α/β values indicate high repair capacity of the cells. It usually characterizes slowly proliferating tissues, in which relatively large cell lifespan is associated with higher chances of DNA damage repair [7]. This renders the long-term effect of radiotherapy on these tissues more sensitive to the dose-per-application than to the length of overall treatment.

To obtain the SF formula, we derive the expression for number of tumor cells at the end of the last treatment for each of the three tumor growth models considered. We assume that tumor regrows between the treatment applications, following one of the growth models. Accordingly, $N(t)$ is repeatedly derived after the application of each treatment. For the exponential tumor growth model (Eq. 3) the SF is derived as follows:

$$N(0) = N_0 \cdot \sigma,$$
$$N(\tau) = N_0 \sigma \cdot e^{r\tau} \cdot \sigma = N_0 \sigma^2 e^{r\tau},$$
$$N(2\tau) = N_0 \sigma^2 e^{r\tau} \cdot e^{r\tau} \cdot \sigma = N_0 \sigma^3 e^{2r\tau},$$
$$\vdots$$
$$N((n-1)\tau) = N_0 \sigma^n e^{(n-1)r\tau}.$$

Hence, the SF after n applications of the irradiation dose (at time $(n-1)\tau$) is:

$$SF_{exp} = \frac{N((n-1)\tau)}{N_0} = \sigma^n e^{(n-1)r\tau}. \tag{8}$$

For the Gompertz model, using Eq. 5 we obtain:

$$N(0) = N_0 \cdot \sigma,$$
$$N(\tau) = K \exp\left[a_g \cdot \ln\left(\frac{N_0 \sigma}{K}\right)\right] \cdot \sigma = K \exp\left[a_g \cdot \ln\left(\frac{N_0}{K}\right) + \ln \sigma(a_g + 1)\right],$$
$$N(2\tau) = K \exp\left\{a_g \cdot \ln\left[\exp\left[a_g \cdot \ln\left(\frac{N_0}{K}\right) + \ln \sigma(a_g + 1)\right]\right]\right\} \cdot \sigma$$
$$= K \exp\left[a_g^2 \cdot \ln\left(\frac{N_0}{K}\right) + \ln \sigma(a_g^2 + a_g + 1)\right],$$
$$\vdots$$
$$N((n-1)\tau) = K \exp\left[a_g^{(n-1)} \cdot \ln\left(\frac{N_0}{K}\right) + \ln \sigma \cdot \sum_{j=0}^{n-1} a_g^j\right] = K \exp\left[a_g^{(n-1)} \cdot \ln\left(\frac{N_0}{K}\right) + \frac{1 - a_g^n}{1 - a_g} \cdot \ln \sigma\right],$$

where $a_g = \exp(-g\tau)$. The SF in this case is:

$$SF_{Gmp} = \frac{K}{N_0} \exp\left[a_g^{(n-1)} \cdot \ln\left(\frac{N_0}{K}\right) + \frac{1 - a_g^n}{1 - a_g} \cdot \ln \sigma\right]. \tag{9}$$

For the power law tumor growth model (Eq. 7) the derivation of the SF is:

$$N(0) = N_0 \cdot \sigma,$$
$$N(\tau) = (A\tau + (N_0 \sigma)^{\frac{1}{3}})^3 \cdot \sigma = (A\tau \sigma^{\frac{1}{3}} + N_0^{\frac{1}{3}} \sigma^{\frac{2}{3}})^3,$$
$$N(2\tau) = (A\tau + A\tau \sigma^{\frac{1}{3}} + N_0^{\frac{1}{3}} \sigma^{\frac{2}{3}})^3 \sigma = (A\tau \sigma^{\frac{1}{3}} + A\tau \sigma^{\frac{2}{3}} + N_0^{\frac{1}{3}} \sigma)^3,$$
$$\vdots$$
$$N((n-1)\tau) = \left(A\tau \sum_{j=1}^{n-1} \sigma^{\frac{j}{3}} + N_0^{\frac{1}{3}} \sigma^{\frac{n}{3}}\right)^3 = \left(A\tau \frac{\sigma^{\frac{1}{3}} - \sigma^{\frac{n}{3}}}{1 - \sigma^{\frac{1}{3}}} + N_0^{\frac{1}{3}} \sigma^{\frac{n}{3}}\right)^3.$$

In this case, the SF at the end of treatment is:

$$SF_{pl} = (\frac{A\tau}{N_0^{1/3}} \cdot \frac{\sigma^{\frac{1}{3}} - \sigma^{\frac{n}{3}}}{1 - \sigma^{\frac{1}{3}}} + \sigma^{\frac{n}{3}})^3. \tag{10}$$

In order to compare the behavior of the three tumor growth models, we choose their parameters so as to have the same initial conditions. For the exponential model, we choose tumor doubling time (t_2) values to be between 10 to 120 days. Thus, the parameter r assumes values of $\frac{ln2}{10}$ to $\frac{ln2}{120}$ $days^{-1}$. For the other two models, tumor doubling time is not constant. It depends on the tumor initial size, as well as on the rate parameters A and g for the power law and Gompertz models, respectively. Hence, we recalculate A and g for each pair of N_0 and r, so that the first doubling time – the time for the tumor to grow from N_0 to $2N_0$ – in the two latter models, will be equal to the constant doubling time in the exponential model:

$$A = \frac{N_0^{1/3} \cdot (2^{1/3} - 1)}{t_2} \tag{11}$$

and

$$g = ^{\frac{1}{t_2}} \ln \frac{\ln (K/N_0)}{\ln (K/N_0) - \ln 2}. \tag{12}$$

In contrast to the exponential growth model, in which the doubling time of the tumor is constant, so that the SF_{exp} values do not depend on N_0, in the other two models the doubling time is changing with tumor size. However, after implementing Eq. 11 into Eq. 10, the value of SF_{pl} also does not depend on N_0. For the Gompertz growth equation (implementing Eq. 12 into Eq. 9), there is still dependence on N_0/K, i.e., on the ratio of tumor size to the limiting carrying capacity of the tumor, K. In this work, we examined Gompertz model for values of the ratio N_0/K being 0.1 and 0.5.

4.3 Simulation of standard radiotherapy treatment: results and discussion

Table 1 shows simulation results for SF values after a conventionally fractionated regimen, that is irradiation of $2Gy$ every 24hrs, for 30 days [21]. Tumor parameters α/β and t_2 were varied, and SF values at the end of treatment were calculated for each of the three growth models. The α/β values were taken between 1 and 20, representing the known range for different tumors in humans [15]. The values of t_2 ranged from 10 to 120 days, as mentioned above. Generally, under all growth laws, the fractionation of the dosage becomes less efficacious with increasing tumor proliferation rate (smaller t_2 values) and decreasing repair capacity (larger α/β). For exponential and Gompertz tumor growth models, our results are comparable to those of McAneney et al. [13]. The small differences are due to a slightly different definition of SF, calculated

by us after n irradiations and $n-1$ intervals in which the tumor repopulation occures, and by [13] after n irradiations and n intervals.

Our results further suggest that if tumor growth obeys power or Gompertz laws, conventionally fractionated regimens are less efficacious than they may be if the exponential model is valid. The reason for that, is the dependance of growth rate on tumor size. Both for power law and Gompertz models, the relative growth rate ($\frac{dN/dt}{N}$) is larger when tumor size is smaller; this stands in contrast to the exponential model, where the relative growth rate is constant. When fractionated irradiation is applied, after each irradiation the tumor shrinks and then regrows during the interval between treatments. In all cases, tumor growth is larger when the interval between treatments is longer. However, in the case of power or Gompertz law, tumor regrowth in the short time immediately following each irradiation is accelerated, because the tumor size is small. This means that as the number of fractions is higher, there are more periods of accelerated tumor growth and this reduces the positive effect of therapy. This was explained by [13] for Gompertz model, but also true for power law model (see Fig. 5).

Evidently, the difference between irradiation effects according to the exponential model and the other two models, as measured in SF values, increases with increasing proliferative capacity of the tumor (smaller t_2 values). How-
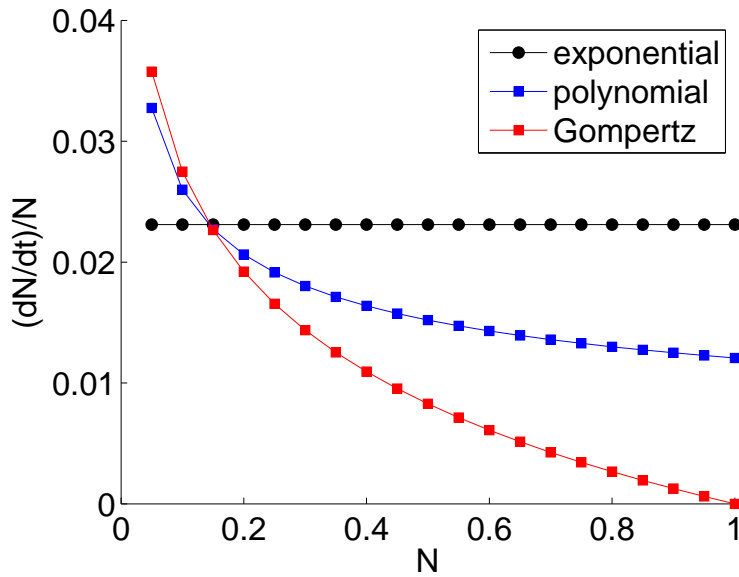

Fig. 5 Comparison of the relative tumor growth rate dependance on tumor size in the different models. Growth rate was calculated for exponential, Gompertz and power law models, according to equations 2, 4 and 6, respectively. Parameter values were taken as $K = 1$, $N_0 = 0.1K$, $t_2 = 30 days$.

ever, this difference is more accentuated as the tumor has higher repair capacity (smaller α/β), for which the long-term effect of radiation on tumor cells is achieved by high irradiation doses per application, rather than by a large number of fractions. This can be seen in Fig. 6, demonstrating the ratio between SF_{pl} and SF_{exp} for different tumor parameters.

Tumor dynamics, obeying the power law growth model is worse than that of tumor with a Gompertz growth of $N_0/K = 0.1$ (resulting in higher SF, see Table 1). However, comparison of SF values for power law model with those for Gompertz model with relatively high initial tumor size ($N_0/K = 0.5$), shows better results for the power law model. In both cases, the ratio between SF_{pl} and SF_{Gmp} is larger when α/β is smaller, and t_2 values are larger (see Figs. 7, 8). This implies that for late-responding tumors, for which the fractionation of radiation dose is less effective, the implications of a power law behavior of the tumor growth become more important. In the case of a power law tumor growth, the results of conventionally fractionated regimen depend on α/β even more than in case of Gompertz growth.

Table 1 Comparison of SF values for conventionally fractionated regimen ($D = 2Gy$, $\tau = 1\ day$, $n = 30\ days$) under different tumor growth models. Survival fraction at the end of treatment was calculated for exponential, power law and Gompertz models (SF_{exp}, SF_{pl} and SF_{Gmp}, respectively) in cases of different tumor parameters α/β and t_2.

α/β	$t2$	SF_{exp}	SF_{pl}	$SF_{Gmp0.1}$[a]	$SF_{Gmp0.5}$[a]
1	30	4.47E-16	5.46E-06	1.17E-13	3.56E-01
	60	3.20E-16	6.84E-07	6.25E-15	1.37E-01
	90	2.86E-16	2.03E-07	2.17E-15	5.10E-02
	120	2.70E-16	8.56E-08	1.26E-15	1.88E-02
5	30	9.83E-08	9.55E-05	1.32E-06	8.94E-01
	60	7.03E-08	1.51E-05	2.86E-07	5.73E-01
	90	6.29E-08	5.54E-06	1.64E-07	3.61E-01
	120	5.95E-08	2.86E-06	1.23E-07	2.27E-01
15	30	2.42E-06	2.52E-04	1.98E-05	1.04E+00
	60	1.73E-06	5.04E-05	5.41E-06	7.27E-01
	90	1.55E-06	2.24E-05	3.38E-06	5.00E-01
	120	1.46E-06	1.35E-05	2.65E-06	3.43E-01

[a] SF_{Gmp} is presented for two different values for tumor size relative to its carrying capacity. $SF_{Gmp0.1}$ and $SF_{Gmp0.5}$ represent results for Gompertz model with $N_0/K = 0.1$ and 0.5, respectively.

5 Conclusions

Even though the possibility that solid tumors grow according to a power law growth function has been suggested in the past [3, 5, 8], and regardless of

the fact that Gompertz or exponential growth have never been convincingly validated experimentally, clinical schedules for cancer therapy are based on the assumptions of Gompertz or exponential tumor growth. These assumptions may not be always true [2, 18]. In this work, we perform *in vitro* and *in vivo* experiments, showing that the possibility that solid tumor growth obeys power law should not be excluded. A simple macroscopic model of tumor growth supports this observation and implies that tumor diameter should grow linearly. This is coherent with our simulation results of a previous model for individual cell behavior, where overall populations dynamics showed power law tumor growth [22]. In our models the dependence of individual cell replication on sufficient resources in the microenvironment results in the inner area of the tumor being in equilibrium, whereas the rim changes dynamically.

Experimental measurements of *in vitro* cancer cell growth in one and two dimensions were in good agreement with the model of linear diameter growth. *In vitro* measurements of tumor size in mouse xenografts also showed unsaturated growth that follows the power growth law. Goodness of fit was compared to that of an exponential growth model, and found to be slightly better in the 3D case and slightly worse in the 2D case. These results indicate that
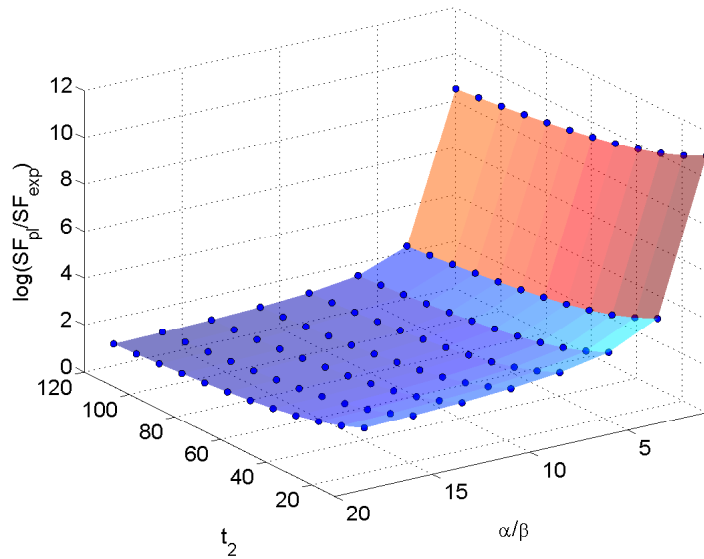

Fig. 6 Dependance of SF on tumor parameters (α/β and the initial doubling time t_2 is different for power law growth model and for exponential growth. The logarithm of the ratio SF_{pl}/SF_{exp} is shown, demonstrating that therapy outcome in case of power law growth becomes worse, relative to the case of exponential model, especially when tumor repair capacity is higher (smaller α/β).

the possibility that in the unsaturated stage of development, solid tumor dynamics obey power law function, is not less realistic than the possibility that they obey the conventionally assumed exponential growth function.

In the past, it has been suggested that the function describing real-life tumor growth is not essential for determining radiotherapy policy [6]. We assert, that the effects of radiotherapy will be significantly different for power law tumor growth. To show that, we predicted the efficacy of a conventionally fractionated regimen of radiotherapy under different assumptions on tumor regrowth in the intervals between irradiations. We calculated the SF of tumor cells after periodic radiotherapy treatment according to each of the three growth models: exponential, Gompertz and the proposed power law. Then, we estimated the SF under different initial conditions and tumor characteristics for each of the models. Comparison between the different growth models showed dramatic differences in tumor response to therapy (orders of magnitude differences in SF values). Moreover, there appeared different trends in the dependence of predicted irradiation efficacy on tumor parameters.

Setting the irradiation schedule to minimize tumor regrowth between treatments could make the difference between failure of treatment and saving the patient's life. In the past, studies of irradiation optimization have
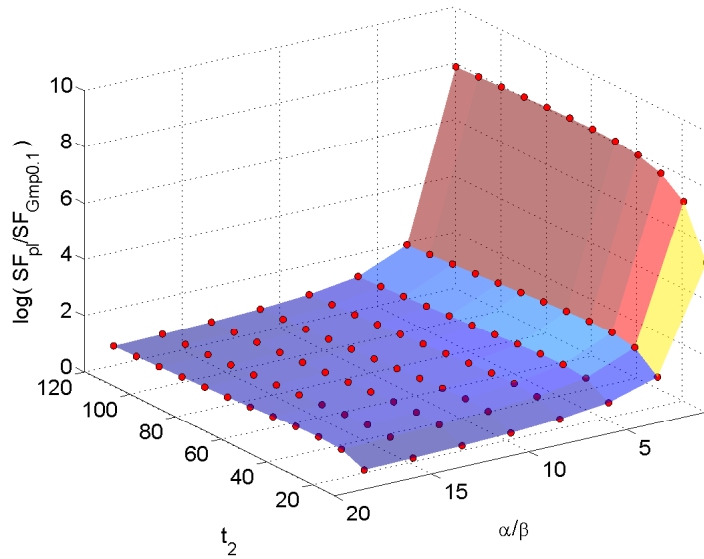

Fig. 7 Dependance of SF on tumor parameters (α/β and the initial doubling time t_2 is different for power law growth model and for Gompertz growth with $N_0/K = 0.1$. The logarithm of the ratio $SF_{pl}/SF_{Gmp0.1}$ is shown, demonstrating that therapy outcome in case of power law growth becomes worse, relative to the case of Gompertz model, especially when tumor repair capacity is higher (smaller α/β).

assumed that tumor growth is exponential [24,25]. Our results, showing both the significance of the tumor growth law for choice of the irradiation schedule, as well as the possibility that tumors grow according to power law, call for further clinical examination of the underlying tumor growth function. If our results are clinically corroborated, it will be worthwhile to search for optimized schedules under the assumption of power law tumor growth. The optimal schedule may be found to be very different than the schedules proposed for the case of exponentially growing tumor, and from the conventionally used schedules.

The possibility of power law growth is usually not considered in the clinical setting, but in this work we showed mathematically and experimentally that it is probable and cannot be dismissed. It is essential to carefully examine this possibility, rather than non-critically embracing the conventional assumptions about tumor growth.
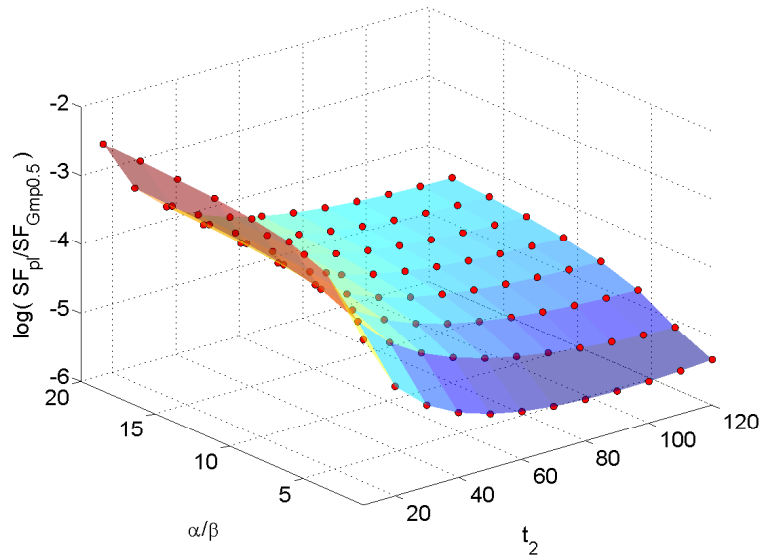

Fig. 8 Same as in Fig. 7 for Gompertz growth with $N_0/K = 0.5$. The logarithm of the ratio $SF_{pl}/SF_{Gmp0.5}$ shows different dependance on tumor parameters. Therapy outcome in case of power law growth becomes worse, relative to the case of Gompertz model, especially when tumor repair capacity is higher (smaller α/β).

6 Materials and methods

6.1 In vitro 1D colony growth experiments

Cells of the breast cancer cell line, MCF-7, were seeded in a 6 cm cell culture dish and cultured in Dulbecco's Modified Eagle Medium (DMEM) at $37°C$ in a humidified atmosphere with 5% carbon dioxide. When cells were grown to 100% confluence a cell-free streak of approx. 1.5 cm width was generated in the middle of the dish by means of a cell-scraper. Afterwards, the proliferation of cells into this cell free area was imaged every 30 min using an Olympus IX81 microscope and the Olympus cellR^R software.

6.2 In vitro 2D colony growth experiments

MCF-7 cells were cultured in DMEM at $37°C$ in a humidified atmosphere with 5% carbon dioxide. Cells were trypsinised and re-suspended in DMEM media. $5\mu l$ and $10\mu l$ ($n = 8$) droplets each containing 10,000 cells were plated onto tissue culture plates and cells allowed to adhere for 3hrs. An additional 10ml of media was then added to plates. Colonies were imaged every 24hrs for 1 week using light microscopy under a 1.25 magnification.

6.3 In vivo xenograft experiments

EMT-6/CTX is a subline of the mouse mammary carcinoma cell line, EMT-6, selected for acquired resistance to cyclophosphamide by multiple exposures to high doses of the drug *in vivo*. EMT-6/CTX cultures were maintained in DMEM supplemented with 10% FCS at $37°C$. 3.0×10^5 EMT-6/CTX cells were injected subcutaneously (s.c.) into the flank of 7-8-week-old male CB6F1 mice. Tumor volume was determined twice a week by an external caliper, the largest longitudinal diameter (length) and the largest transverse diameter (width) were determined. Tumor volume was calculated by the formula: $Tumor\ volume = 0.52 \times length \times width^2$.

6.4 Computational methods

For 1D colony growth, we used the linear radius growth model, $R(t) = At + B$, where A is the radial growth rate and B is the initial colony size. We numerically fitted these parameters to the experimental data for each of the experi-

ments. For 2D (colony area) experiments, we assumed circular structure and fitted $\pi R(t)^2$ to find the parameter A of radial growth rate accordingly. For 3D (tumor volume) measurements, we assumed tumor structure was spherical, and fitted $\frac{4\pi}{3} R(t)^3$. In all cases, the fitting was done numerically using Matlab local search algorithm (fminsearch). For comparison, the same data was fit to an exponential model $R(t) = A \exp(\lambda t)$, where the value of λ was found using the same local search algorithm (fminsearch) in Matlab.

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