Implications of a simple mathematical model to cancer cell population dynamics

A. L. Garner*, Y. Y. Lau*, D. W. Jordan*, M. D. Uhler*·†
and R. M. Gilgenbach*

*Bioelectromagnetism Laboratory, Department of Nuclear Engineering and Radiological Sciences,
University of Michigan, Ann Arbor, MI and †Molecular and Behavioural Neuroscience Institute,
University of Michigan, Ann Arbor, MI, USA

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Abstract. Recent research in cancer progression and treatment indicates that many forms of cancer arise from the development of a small subpopulation of abnormal cancer stem cells (CSCs) that promote cancer growth and spread. Many potential treatments preferentially interact with cells at certain stages of the cell cycle by either selective killing or halting the cell cycle, such as intense, nanosecond-duration pulsed electric fields (nsPEFs). Simple mathematical models of unfed cancer cell populations at the plateau of their growth characteristics may estimate the long-term consequences of these treatments on proliferating and quiescent cell populations. Applying such a model with no transition from the quiescent to proliferating state shows that it is possible for the proliferating cell population to fall below 1 if the quiescent cell population obtains a sufficient competitive advantage with respect to nutrient consumption and/or survival rate. Introducing small, realistic transition rates did not appreciably alter short-term or long-term population behaviour, indicating that the predicted small cell population behaviour (< 1 cell) is not an artefact of the simpler model. Experimental observations of nsPEF-induced effects on the cell cycle suggest that such a model may serve as a first step in assessing the viability of a given cancer treatment *in vitro* prior to clinical application.

INTRODUCTION

Cancer cell populations consist of a combination of proliferating, quiescent and dead cells that determine tumour growth or cancer spread based on surrounding environmental conditions (Freyer & Sutherland 1986; Luciani *et al.* 2001). Moreover, recent research suggests that many cancers originate from one abnormal cell or a small subpopulation of abnormal cells that maintain tumour growth (Reya *et al.* 2001), including leukaemia (Warner *et al.* 2004), breast cancer (Dick 2003; Dontu *et al.* 2003) and nervous system tumours (Singh *et al.* 2004a,b). Targeting these cancer stem cells (CSCs) is vital because they display many of the same characteristics as

Correspondence: Professor Y. Y. Lau, Nuclear Engineering and Radiological Sciences, Cooley Bldg, 2355 Bonisteel Blvd, Ann Arbor, MI 48109-2104, USA. Tel.: +734 764-5122; Fax: +734 763-4540; E-mail: yylau@umich.edu

healthy stem cells, including the capability of initiating new tumours following long periods of remission (Behbod & Rosen 2004). This increased understanding of the cancer mechanism could have a significant impact on cancer treatment strategies as it emphasizes the importance of treating a specific subset of the cancer cells at a specific stage of development rather than using non-selective cytotoxic agents on as large a population of dividing cells as possible (Jones *et al.* 2004; Massague 2004; Sawyers 2004).

A prime example of the importance of proper cellular targeting is the recent case of the new drug, imatinib, which has been used to treat chronic myeloid leukaemia (CML) (Jones *et al.* 2004). Using interferon, the typical treatment for CML for decades, was slow acting, but resulted in few relapses when successful. The desire to rapidly treat patients who succumb prior to interferon's effect led to the development of imatinib, which acted more rapidly than interferon in preliminary clinical studies. Unfortunately, recent studies indicate that many patients treated with imatinib relapsed, possibly because imatinib is highly toxic to differentiated CML progenitors while CML *stem cells* may be resistant to the drug (Jones *et al.* 2004). Current research has provided some quantitative insight into the *in vivo* kinetics of CML and further illustrated that imatinib is a potent inhibitor of differentiated leukaemia cells existence while not depleting the stem cells (Michor *et al.* 2005). Thus, although interferon acted more slowly than imatinib, it more directly targeted the stem cells, minimizing the potential for a relapse.

Other modalities that impact different cell subpopulations are being investigated for cancer treatment. For instance, recent advances in pulsed power technology have enabled researchers to study the biological effects of ultrashort (pulse durations from 10 to 300 ns), high intensity (75–300 kV/cm) electrical pulses (Schoenbach *et al.* 2004). Because of their short pulse duration, these nanosecond lasting pulsed electric fields (nsPEFs) charge the membranes of intracellular organelles, paving the way for manipulation of cellular function (Schoenbach *et al.* 2001, 2004). Of particular interest are observations that nsPEFs can induce apoptosis in cancer cells both *in vitro* and in tumours (Beebe *et al.* 2002, 2003). Moreover, nsPEFs have specific, genotoxic effects on cells of certain lines in culture while not impacting others, potentially presenting a way for selective cancer treatment (Stacey *et al.* 2003). From a cell population perspective, workers have observed that applying fewer than twenty pulses caused a minor decrease in cell survival after 24 h, whereas much longer trains of nsPEFs caused a sharp drop in cell survival with minimal thermal significance (Pakhomov *et al.* 2004). The target and pulse shape can have a critical impact on the result, as illustrated by recent observations that show that prolonged exposure to ultra-wideband pulses can promote preneoplastic mammary epithelial cell proliferation (Sylvester *et al.* 2005). These results, in addition to our own recent work on electrical pulse-induced effects (Garner *et al.* 2004; Jordan *et al.* 2004), have motivated our current study on cell population modelling, particularly predicting the behaviour of proliferating and quiescent cells after an external stimulus, such as the multiple nsPEFs studied by Pakhomov and colleagues.

An increased understanding of the roles of the different types of cells in cancer development and treatment, as well as the immense potential of stem cells in regenerative treatment, has raised interest in constructing appropriate mathematical models for the proliferation of these types of cell (Deasy *et al.* 2003). One approach is to use a discrete model, starting from the stem cell population, considering self-renewal and differentiation until the cells are either fully differentiated or dead (Tomlinson & Bodmer 1995). This type of model is useful for examining how changes in self-renewal and apoptotic rates lead to changes in the haematopoietic cell cycle and ultimately cancer (Al-Hajj & Clarke 2004; Attar & Scadden 2004; Cheng 2004), as well as potentially lending insight into possible treatments (De Graaf *et al.* 2004). Particularly interesting is Tomlinson and Bodmer's (1995) observation that the cell population only reaches a steady state when half the stem cells undergo self-renewal, with exponential growth or decay of the

semi-differentiated and differentiated cell populations occurring for small perturbations in this fraction (Tomlinson & Bodmer 1995). However, their model is more suited to considering cell cycle changes for cells in the body as they differentiate from stem cells while we are currently investigating cells in suspension, which do not pass through these developmental stages.

Because of the importance of cell proliferation in considering changes in a cancer cell population, it is natural to speculate on models that focus on these areas together, particularly when the cancer cells are treated with a therapeutic agent. In one such model, untreated growth of breast and ovarian cancer cell lines was compared to their growth after treatment with the antimitotic agent, curacin A, which alters the rate of cell proliferation (Kozusko *et al.* 2001). The model used in this study was continuous and included five coupled differential equations that considered the S and M phases of cells with and without drugs and an equation representing drug concentration in the *in vitro* medium external to the cells (Kozusko *et al.* 2001). A further model analysed apoptotic effects caused by treatment with anticancer drugs using a multicompartment model of the dividing-cell cycle with each compartment corresponding to a distinct phase (Basse *et al.* 2004). More generally, one can consider cell cycle progression by including stochastic effects in the models to represent inherent fluctuations (noise) in complex biological systems (Roeder & Loeffler 2002; Steuer 2004; Tyrcha 2004).

While these models often lend insight, they are frequently complicated and derived for specific conditions. In the case of a suspension of malignant cells exposed to nsPEFs and/or chemotherapeutic agents, simpler, more general models may be considered as a starting point (Sherley *et al.* 1995; Solyanik *et al.* 1995; Deasy *et al.* 2003). Deasy's model, adapted from Sherley's, starts from the initial assumption that proliferating cells grow exponentially if all the cells divide ($N = N_0 2^{t/DT}$, where N is the number cells, t is the time, N_0 is the initial number of cells, and DT is the division time) and can be adapted by assuming that only a certain fraction, α , actually undergoes cell division (Sherley *et al.* 1995). Deasy extended this model to include cell death, M , which in our case could represent the cytotoxic effect of nsPEFs or chemotherapy. Although this model is simple and easy to apply, the actual experimental measurement of M requires counting the total number of dead cells virtually continuously, which is manpower-intensive experimentally. The continuous model developed by Solyanik *et al.* (1995) estimates the behaviour of proliferating and quiescent cell populations based on approximately 10 measurements of the total cell population over approximately 1 week, which is more practical experimentally. Solyanik's model utilizes two coupled, nonlinear differential equations with the final cell behaviour dependent upon initial total cell number and the ratio between proliferating and quiescent cells. It is then straightforward to analyse the resulting growth and steady-state behaviour of these cell populations over time. This type of model provides a means to assess the effectiveness of a given treatment *in vivo* before continuing to clinical studies. Thus, we further investigate the analysis conducted by Solyanik and colleagues, particularly focusing on long-term behaviour predicted by the equations in light of recent interest in CSC-targeted cancer treatment.

In the Materials and Methods section, we outline the development of the model. We then conduct numerical analysis of some sample cases and discuss potential implications and applications of the model.

MATERIALS AND METHODS

Figure 1 shows a block diagram of the system that Solyanik *et al.* introduced in their work (1995). They assumed the following:

Figure 1. Block diagram of the mathematical model (Solyanik *et al.* 1995). Here, $x(t)$ is the number of proliferating cells at time t , $y(t)$ is the number of quiescent cells at time t , b is the rate of the cell division of the proliferating cells, d is the rate of cell death of the resting cells (per day) and $Q(x, y)$ and $P(x, y)$ describe the intensity of cell transition from the quiescent to proliferating state and visa verse (per day), respectively.

- (1) The cancer cell population consists of proliferating and quiescent (resting) cells.
- (2) The cells can lose their ability to divide under certain conditions and therefore transit from the proliferating to the resting state.
- (3) Resting cells can either return to the proliferating state or die.
- (4) Cell death is irreversible.

The growth kinetics can therefore be expressed in terms of the following two coupled non-linear differential equations:

$$\begin{aligned} x'(t) &= bx(t) - P(x,y)x(t) + Q(x,y)y(t) \\ y'(t) &= P(x,y)x(t) - Q(x,y)y(t) - dy(t), \end{aligned} \quad (1)$$

where $x(t)$ is the number of proliferating cells at time t , $y(t)$ is the number of quiescent cells at time t , b is the rate of cell division of the proliferating cells, d is the rate of cell death of the resting cells (per day) and $Q(x,y)$ and $P(x,y)$ describe the intensity of cell transition from the quiescent to proliferating state and vice versa (per day), respectively. Note that one could also validly define $x(t)$ and $y(t)$ as the number densities of proliferating cells and quiescent cells, respectively; however, we refer to these quantities in terms of absolute cell number to be consistent with the nomenclature used by Solyanik *et al.* (1995). Based on the observation that cancer cells proliferate in the presence of sufficient biological and physical factors, they assumed that $P(x,y)$ must depend on the number of cells present and, in simplest form, can be written as

$$P(x,y) = c[x(t) + ay(t)], \quad (2)$$

where a is a dimensionless constant that measures the relative nutrient uptake by resting and proliferating cells, and c depends on the intensity of consumption by proliferating cells and gives the magnitude of the rate of cell transition from the proliferating stage to the resting stage in per cell per day (i.e. cell $\times$ per day). Solyanik *et al.* (1995) pointed out that transition from the quiescent to the proliferating state was more complex. One would generally assume that increasing the number of proliferating cells would decrease the intensity of this transition; however, a decrease in the number of proliferating cells can also lead to a decrease in the transition (Wallen *et al.* 1984a,b). Based on these observations, one can assume that $Q(x,y)$ is non-monotonous with respect to x so that it increases with increasing x up to a certain point and then decreases as x , the number of proliferating cells, becomes very large. Including the time dependence, $Q(x,y)$ can be written as

$$Q(x,y) = \frac{Ax(t)}{1 + Bx^2(t)}, \quad (3)$$

where A represents the initial rate of increase in $Q(x,y)$ at small x and A/B represents the rate of decrease in $Q(x,y)$ when x becomes larger.

Solyanik *et al.* then substituted equations 2 and 3 into equation 1 and studied the resulting steady states and stability. However, they assumed that the intensity of cell transition from the quiescent state to proliferation was small compared with the other processes when they applied this model to experimental data, allowing them to neglect $Q(x,y)$ and, thus, eliminate A and B . Because they neglected $Q(x,y)$ in their analyses, it is informative to consider the characteristics of the steady states with $Q(x,y) = 0$.

Substituting equation 2 for 1 and considering $Q(x,y) = 0$ yields

$$\begin{aligned} x'(t) &= bx(t) - c[x(t) + ay(t)]x(t) \\ y'(t) &= c[x(t) + ay(t)]x(t) - dy(t). \end{aligned} \quad (4)$$

Equation 4 has steady states at $(x, y) = (0, 0)$ and $[db(ab + d)^{-1}/c, b^2(ab + d)^{-1}/c]$ where the first term in parenthesis represents the x_{ss} and the second represents y_{ss} . Linear stability analysis of these steady states shows that the zero steady state is a saddle point and the nontrivial steady state is stable. Particularly interesting is that x_{ss} depends on d , the rate of death of the quiescent cells. Thus, for small d , the quiescent cells will die at a lower rate and x_{ss} will decrease because the quiescent cells will have a relative competitive advantage. In our analysis, we pay special attention to the cases where there is less than one proliferating cell, which means that the total cell population must exponentially decay to zero in the case of $Q(x,y) = 0$. We next non-dimensionalize the model to further examine such effects of these parameters on model behaviour.

NON-DIMENSIONALIZATION

To simplify the notation and reduce the number of parameters, we introduce the following dimensionless parameters:

$$\bar{x} = \frac{xc}{b}, \bar{y} = \frac{yca}{b}, \bar{t} = bt. \quad (5)$$

After dropping bars for notational convenience, the non-dimensional system is

$$\begin{aligned} x'(t) &= x(t) - x(t)[x(t) + y(t)] \\ y'(t) &= ax(t)[x(t) + y(t)] - ry(t), \end{aligned} \quad (6)$$

where $r = d/b$ and we additionally define $z(t)$ as the non-dimensional total number of cells, $z(t) = x(t) + y(t)$. Note that we have reduced this system from four independent parameters to two, where one of the parameters, r , is the ratio of the death rate to the birth rate ($r = d/b$). Equation 6 has x null clines given by $x = 0$ and $x + y = 1$ and y null clines given by $y = 0$ and $y = (a/r)x(x + y)$, giving steady states at $(0, 0)$ and $[r/(a + r), a/(a + r)]$. As in the dimensional case in previous discussion, the zero steady state is a saddle point and the nontrivial steady state is stable for physically realistic conditions ($a > 0, r > 0$). Particularly interesting is that the steady state in this form shows that only two parameters, a and r , are important in determining the overall behaviour of the cell populations. In essence, the ratio of cell death and birth rates (r) and the ratio of the nutrient consumption intensities of the cell populations (a) determine the final steady-state populations. These results agree with the analyses of Solyanik *et al.* but the

Table 1. Model parameters for the mouse mammary cell lines 66, 67, and 68H (Solyanik *et al.* 1995). In all cases, there were initially 7×10^4 proliferating cells and 3×10^4 quiescent cells

Cell line	b (per day)	c (10^{-7} cell $^{-1}$ per day)	a	d (per day)
66	1.56	2.5	0.6	0.117
67	1.36	2.0	1.55	0.7
68H	1.19	2.5	3.1	0.0007

mathematics involved is simplified here by omitting $Q(x,y)$. For cases where $Q(x,y)$ cannot be neglected, we obtain the following nondimensional system:

$$\begin{aligned} x'(t) &= x(t) - x(t)[x(t) + y(t)] + \frac{hx(t)y(t)}{1 + kx^2(t)} \\ y'(t) &= ax(t)[x(t) + y(t)] - ry(t) - \frac{hx(t)y(t)}{1 + kx^2(t)}, \end{aligned} \quad (7)$$

where $h = A/ac$ and $k = Bb^2/c^2$. Here, h represents a growth factor that preferentially shifts cells from the quiescent to proliferating state, whereas k represents a slightly moderating effect. For simplicity, we set $k = 1$ and vary h to represent changes in the effects of these growth factors, as $Q(x,y)$ is more strongly dependent on h .

RESULTS

First, we considered the sample cases studied by Solyanik *et al.* involving the growth of mouse mammary tumour cells (66, 67, and 68H) that were not provided with additional nutrients during the study (i.e. unfed) (Wallen *et al.* 1984a,b). We examined the behaviour of the systems over longer time frames using the parameters in Table 1 to verify the existence of the non-trivial steady state predicted by equation 6. We verified this behaviour for cell lines 66 and 67; however, cell line 68H eventually decayed to the trivial steady state when the non-dimensional system was used, as shown in Fig. 2.

Solyanik *et al.* considered the fraction of proliferating and quiescent cells as a function of time for these three cell lines and noted that there was a much higher fraction of quiescent cells; however, they did not explicitly consider the absolute number of cells. This is critical for cell line 68H, where $x(t)$ becomes less than one within 12 days in the dimensional case ($x < x_{\text{crit}} = c/b$ in the dimensionless case), meaning that physically, there are no proliferating cells remaining. Based on their assumption that $Q(x,y) = 0$, it is impossible to supply more proliferating cells, meaning that the supply of quiescent cells is gone and both $y(t)$ and $z(t)$ will eventually decrease to zero at a rate dependent upon r . The solution for the total number of cells approaches the non-trivial steady state in the dimensional case because $x(t)$ never becomes zero numerically, meaning that the equations can recover and still provide a non-zero quantity of cells. However, in the non-dimensional case, $x(t)$ becomes vanishingly small for a long time, thus putting the solution on the stable manifold of the saddle point (Strogatz 1994), causing $y(t)$ and, thus, $z(t)$ to eventually reach zero. While this behaviour essentially arises as a result of a numerical artefact and can be eliminated by altering differential equation solvers or working precision, this result agrees with the physical intuition that the presence of less than one proliferating cell is equivalent to having no proliferating cells.

Figure 2. Extrapolation of the non-dimensional total cell population of cell line 68H (Wallen *et al.* 1984a,b) on a logarithmic scale versus nondimensional time based on the parameters obtained by Solyanik *et al.* (1995). Note that the total cell population decreases exponentially over a long timescale.

Obviously, this behaviour is contingent upon $Q(x,y)$ being negligible. Although Solyanik *et al.* argued that $Q(x,y)$ was likely to be small because of the large duration of growth delay and excellent agreement between the model and experiment, this does not necessarily completely eliminate the effect, particularly at small x . One would speculate that the effect of $Q(x,y)$ is small and will likely raise $x_{\min}$, the minimum number of proliferating cells, $x(t)$, for $t > 0$, and/or increase the time it takes to reach $x_{\min}$, which is not necessarily zero. As $x_{\min}$ becomes small, it becomes important to establish the significance of small $Q(x,y)$ on $x(t)$.

Figure 3a shows $z(t)$ for the experimentally observed time frame for cell line 68H with $k = 1$ and $h = 0.00005, 0.0005, 0.005, 0.05, 0.5$, and 1 . For $h < 0.05$, we observed little change in the behaviour of $z(t)$ compared to the experimental data; however, for $h > 0.5$, we noted a distinct difference in curve shape. This suggests that $0.05 < h < 0.5$ causes observable cell transition from the quiescent state to the proliferating state over the experimentally observed time frame. Physiologically, this implies that there is a critical quantity of growth factor that will cause a build up of proliferating cells instead of death; however, in most cases, this growth factor quantity is likely to be small and can be neglected. Figure 3b shows the behaviour of $y(t)$ over this same time frame. Although the magnitude of $y(t)$ is initially lower than that of $z(t)$, we note that it quickly approaches $z(t)$ as $x(t)$ becomes small. This is expected because $x(t)$ decreases dramatically after initially increasing, as shown in Fig. 3c.

To observe the effect of these perturbations over a longer timescale, specifically to examine the effects on $x_{\min}$, we plot $x(t)$ over a longer time frame in Fig. 3c. As observed for the experimental time frame, $h < 0.05$ has little effect on the time to reach $x = x_{\text{crit}} = c/b$, which corresponds to one proliferating cell remaining (i.e. $x = 1$ in dimensional units). Interestingly, even though $h = 0.5$ has a noticeable effect on $z(t)$ in the experimental time frame, $x(t)$ still decreases below x_{crit} after a slightly longer time delay. Raising h to 1.0 dramatically effects $z(t)$ over the range of experimental data and causes $x(t)$ to reach a non-zero steady state without ever approaching x_{crit} . At this point, h is large enough so that the steady-state behaviour must be determined using the equations from Solyanik *et al.* instead of the simpler equations derived above.

We next turned our attention to a hypothetical case in which $x_{\min} \sim x_{\text{crit}}$. Here, we used the parameters of cell line 68H from Table 1 except we set $d = 0.066$ per day to give $x_{\min} \sim x_{\text{crit}}$. Figure 4 summarizes the results from carrying out the same exercise as previously mentioned for this borderline case. Figure 4a,b show $z(t)$ and $y(t)$, respectively, for different h over a typical experimental time frame. A slight effect that probably would be indistinguishable from experimental variation occurs for $h = 0.05$. Raising $h \geq 0.5$ leads to a dramatic change in the behaviour of $z(t)$ and $y(t)$, as well as $x_{\min}$, as shown in Fig. 4c. Although noticeably different from the curves for

Figure 3. Non-dimensional (a) total cell population $[z(t) = x(t) + y(t)]$ (b) quiescent cell population $[y(t)]$, and (c) proliferating cell population $[x(t)]$ plotted on a logarithmic scale versus dimensionless time over the experimental time frame for cell line 68H. Here, $x(0) = 0.0147059$, $y(0) = 0.0195378$, $a = 3.1$, $r = 5.88 \times 10^{-4}$, $k = 1$, and $h = 0.0005, 0.005, 0.05, 0.5, 1$ from the bottom curve to the top curve. (a) Note that $Q(x,y)$ has little impact on $z(t)$ for $h \leq 0.05$, whereas it has a noticeable impact for $h \geq 0.5$. (b) Note that $Q(x,y)$ has little impact on $y(t)$ for $h \leq 0.05$, whereas it has a noticeable impact for $h \geq 0.5$. (c) The horizontal line at $x = x_{\text{crit}} = c/b = 2.1 \times 10^{-7}$ represents a population of one proliferating cell. Note that $Q(x,y)$ has little impact on the time to reach x_{crit} until $h = 0.5$. Note that $h = 1$ causes $x_{\text{min}} > x_{\text{crit}}$.

Figure 4. Non-dimensional (a) total cell population $[z(t) = x(t) + y(t)]$ (b) quiescent cell population $[y(t)]$, and (c) proliferating cell population $[x(t)]$ plotted on a logarithmic scale versus dimensionless time over the experimental timeframe for a hypothetical cell line with $x_{min} \sim x_{crit}$ for $h = 0$. Here, $x(0) = 0.0147059$, $y(0) = 0.0195378$, $a = 3.1$, $r = 0.0554622$, $k = 1$, and $h = 0.0005, 0.005, 0.05, 0.5, 1$ from the bottom curve to the top curve. (a) Note that $Q(x,y)$ has little impact on $z(t)$ for $h \leq 0.05$, whereas it has a noticeable impact for $h \geq 0.5$. (b) Note that $Q(x,y)$ has little impact on $y(t)$ for $h \leq 0.05$ while it has a noticeable impact for $h \geq 0.5$. (c) The horizontal line at $x = x_{crit} = c/b = 2.1 \times 10^{-7}$ represents a population of one proliferating cell. Note that when $x \sim x_{crit}$, $h = 0.5$ causes $x_{min} > x_{crit}$ while $h = 1.0$ causes x to increase by approximately five to six orders of magnitude.

$h < 0.05$, the minimum for $h = 0.5$ is only several times larger than x_{crit} , meaning that it corresponds to approximately tens of cells. Thus, even for a relatively large transition from the quiescent to proliferating state, the end result is still a small number of cells that could, statistically, be small enough to lead to population decay over a long period of time. Raising h to 1.0 has a major effect on $x(t)$, causing x to be approximately six orders of magnitude larger than for $h = 0.5$.

DISCUSSION

Although statistical fluctuations in cell population are likely to impact upon the values of $x(t)$, $y(t)$, and $z(t)$ predicted by this model, particularly for smaller population sizes, it is interesting to note that although a cell population, such as cell line 68H, appears to be in a plateau growth phase, it is really at a point where cell population decay is about to slowly take place. One of the major advantages of this type of model is that it enables predictions of cell population behaviour that would be difficult to conduct experimentally, such as maintaining an unfed cell line population and conducting counts over a period of time longer than approximately 2 weeks. Experimentally, Wallen *et al.* observed that > 98% of the cells present excluded trypan blue, a common stain used in cell culture viability analyses that is excluded from a living cell with an intact cell membrane but is permeable (and identifiable using a light microscope) to a dead cell, after 2 weeks in plateau (1984a,b). Although the cells were predominantly alive, a small fraction was still proliferating, as indicated by a decrease in colony-forming ability because of the increasing fraction of quiescent cells (Wallen *et al.* 1984a,b). Naturally, some cells will continue to die, but the overall cell population will be maintained as long as a sufficient fraction of proliferating cells remains.

From a cancer-treatment perspective, being able to predict and minimize proliferating cell populations is critical. Cancer treatments that preferentially interact with CSCs, or the proliferating cancer cells, should be more durable, or less susceptible to relapses. This model suggests that treatments that lower r (i.e. raise b and/or lower d) will decrease the minimum number of proliferating cells. This occurs because lowering r gives quiescent cells a competitive advantage, as it lowers the decay rate of $y(t)$, which raises the growth rate of the quiescent cells compared to the proliferating cells. Therefore, the quiescent cells will take more resources from the proliferating cells, causing their number to drop, decreasing x_{min} . Although likely more difficult to control, raising a would have the same type of effect, causing the quiescent cells to have a competitive advantage over the proliferating cells in obtaining limited nutrients. From the steady-state equations, we also observe that changing a and r in this fashion causes the steady-state number of proliferating cells to decrease and the steady-state number of quiescent cells to increase. In cases where x is not driven below x_{crit} , some proliferating cells would remain, but their number would be much smaller than prior to treatment. Moreover, this could mean that subsequent treatments targeting the smaller population of proliferating cells could be used to continue to drive the proliferating cell population towards and below x_{crit} .

This type of model could be useful in simple *in vitro* experiments when validating the success of a given treatment prior to clinical studies. By fitting a week or two of experimental data to this model, it would be possible to estimate the long-term behaviour of the proliferating and quiescent cell populations to determine the likelihood of treatment success. For example, recent studies concerning nsPEFs have shown changes in cell population number (Pakhomov *et al.* 2004) and numbers in the cell cycle (Stacey *et al.* 2003) when appropriate pulse parameters were used. Particularly interesting are observations that nsPEF exposure caused cells to exhibit lower mitotic indices and lower survival rates, indicating that the surviving cells may accumulate at a

fixed point in the cell cycle before mitosis (Stacey *et al.* 2003). Genomic studies by Wang *et al.* lend credence to these observations by showing that genes involved in mitosis are down-regulated, whereas genes responsible for cell cycle arrest are up-regulated, which would lower the mitotic index and cause cells to pause in the quiescent state (Wang *et al.* 2005). Thus, nsPEFs appear to raise the length of the mitotic cycle, which lowers b , and increase cell death rate, which raises d . This raises r , which raises $x_{\min}$; however, we still need to determine the impact of the nsPEFs on a . Because a measures the competition between proliferating and quiescent cells for limited resources, and more cells are accumulating in the cell cycle prior to mitosis (i.e. they are quiescent), it appears likely that a would rise. Thus, the overall success of the treatment would depend on whether this rise in a would be sufficient to overcome the rise in r and drive $x_{\min} < x_{\text{crit}}$. Also unknown at present is the impact of the nsPEFs on the initial fractions of proliferating and quiescent cells at initial growth. When the strongest effects of nsPEFs on cell populations occur, the population initially decreases before recovering and approaching standard growth (Pakhomov *et al.* 2004). Based on Stacey's work, it seems probable that nsPEFs would increase the fraction of quiescent cells at the point of population growth, but this still remains to be quantified or estimated for specific cases before this model can be applied. Moreover, $Q(x,y)$ cannot necessarily be immediately neglected because nsPEFs prevent a group of cells from entering the mitotic pathway, and it is possible that these cells could subsequently become proliferating. The recent observation that ultra-wideband pulses may even stimulate proliferation of certain cells under certain conditions gives another reason for careful consideration when applying this model to experimental data (Sylvester *et al.* 2005). Nevertheless, neglecting $Q(x,y)$ as a first order approximation when a large fraction of cells initially dies and there is no indication of significant transition to proliferation over a long timeframe is likely to be valid.

The results of this model for *in vitro* data could also benefit current research involving nsPEF treatment of murine tumours (Nuccitelli *et al.* 2005). As with cell suspensions, tumours contain a combination of proliferating, quiescent, and dead (necrotic and apoptotic) cells (Freyer & Sutherland 1986). Applying nsPEFs to tumours causes apoptosis (Beebe *et al.* 2002, 2003) and possible destruction of the surrounding vasculature (Nuccitelli *et al.* 2005). Thus, predicting and quantifying proliferating and quiescent cell population changes induced by nsPEFs may elucidate some of the cellular effects, whereas other models examining tumour growth (Garner *et al.* 2005) and anti-angiogenesis (Anderson & Chaplain 1998) may be useful for interpreting the overall effect on the tumour. This relationship between models and biological processes becomes even more evident, as recent experimental evidence has established the importance of angiogenesis in the spread of haematopoietic cancer, in addition to solid tumours (Ribatti *et al.* 2004).

CONCLUSION

We have analysed a simple cancer cell population model to estimate long-term behaviour of quiescent and proliferating cells. Unfed cell populations initially grow exponentially before reaching a plateau in their growth curves (Wallen *et al.* 1984a,b). Mathematical modelling of these data have shown that the quiescent cell population was much higher than the proliferating cell population (Solyanik *et al.* 1995); however, Solyanik and colleagues never noted that the model predicted that the proliferating cell population decreased below one for one cell line. By concentrating on the behaviour of this model at small populations, particularly below one, it is possible to examine the effects of various treatments and possible population recovery. Non-dimensionalizing Solyanik's equations permitted us to reduce the number of independent

parameters to two and further concentrate on the effects of changes of these primary parameters to population behaviour. Applying this model to Wallen's data shows that if the ratio of quiescent cell death rate to proliferating cell birth rate (r) is sufficiently smaller than the quiescent cells' relative advantage in nutrient consumption (a), then the proliferating cell population can drop rapidly in a short period of time, resulting in the elimination of the proliferating cell population. Although statistical fluctuations could lead to some proliferating cells in these cases, it is also possible in cases predicting small proliferating cell populations, that no proliferating cells are present.

This population behaviour could provide a means to measure the effectiveness of various cancer treatments *in vivo* prior to clinical applications. Specifically, treatments that target the proliferating cells *in vivo* could ultimately be developed as selective treatments for CSCs. Although the presence of a 'magic bullet' for attacking CSCs is unlikely, it is possible that combining various modalities could accomplish this for certain cancers (Behbod & Rosen 2004). Mathematically, such treatments would succeed by somehow specifically targeting the cancer stem cell population in such a way that the quiescent population obtains a competitive advantage, either through increasing a or decreasing r . It is also important to recognize that although destroying the proliferating cells is crucial to eliminating the CSCs, quiescent CSCs can survive for long periods of time and may also play a role in cancer relapses (Reya *et al.* 2001; Taipale & Beachy 2001). This would mean that at some point, $Q(x,y)$ becomes sufficiently large such that $x > x_{\text{crit}}$ and $x'(t) > 0$ so that the proliferating population is re-established. Including such behaviour in the current model would likely require incorporating statistical and/or stochastic processes and would likely be more applicable to clinical trials than the current *in vitro* analysis. A model that outlines the development of cell differentiation *in vivo*, such as performed by Tomlison and Bodmer, would be more applicable to the long-term effects of cancer treatment *in vivo* (Tomlinson & Bodmer 1995).

A possible example with nsPEF pulses shows that although they cause an increase in r as a result of increased mitotic cycle length and an increased rate of cell death, they also cause more cells to remain in the pre-mitotic division stage (Stacey *et al.* 2003). This could raise a because of the increased consumption of nutrients by these quiescent cells, resulting in proliferating cell population decrease if this advantage is sufficient. Although population studies have been conducted (Pakhomov *et al.* 2004), further studies concerning the effect on nsPEFs on the sub-populations would enable mathematical modelling to predict long-term treatment consequences, which may be applicable to understanding cell population behaviour in ongoing research on tumour treatments (Nuccitelli *et al.* 2005).

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