# A simple new alternative to the linear-quadratic model (and where the LQ model comes from)

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**Abstract**

I present a new dose-survival equation for fitting clonogenic assay data collected for cancer cells exposed to radiation. It is a single-target model. The short form of the equation, applicable to normally radiosensitive cells, has three parameters. In contrast to previous target models, this one recognizes that (1) the state a cell is in, when radiation is administered, determines the number of strikes needed to sterilize it (cause it to lose the property of being indefinitely clonogenic) and that (2) different cells in a clonogenic assay will generally be found in different states, unless pains were taken to synchronize them. Excellent fits to published data sets are obtained under the hypothesis that there are *two* underlying cellular states which differ in their sensitivity to low-LET radiation. I call these "state R" and "state Q." A second hypothesis is then advanced, regarding the time-duration of states R and Q, which explains the empirical observation that rapidly proliferating cancer cells have a straighter dose-survival relationship, while slowly proliferating cancer cells and normal cells have a curvier one. The LQ model can be derived from the new dose-survival equation by taking a Taylor expansion; this yields formulas for $\alpha$ and $\beta$ in terms of the new model's parameters. The resulting formula for $\alpha/\beta$ reveals why this ratio grows larger as replication rate increases. The new dose-survival equation enables us to decompose $SF(D)$ (the fitted curve) into theoretical state-R and state-Q dose-survival curves. If the more radiation-resistant of these, generally the state-Q curve, is used to select a fraction size for fractionated radiotherapy, cell cycle phase is eliminated as a variable in treatment outcome. The decomposition may also enable us to standardize the results reported for clonogenic assays, which in raw form depend upon the conditions in irradiated cell culture (percent confluence, medium used, etc.). Finally, the new dose-survival equation provides a convention for classifying cells as radioresistant. In the *Discussion*, I offer a hypothesis regarding what states R and Q actually represent, and propose a novel hypothesis for the mechanism behind the "oxygen enhancement ratio" phenomenon. All results are obtained using only high-school mathematics and this paper is accessible to everyone.

## 1. Introduction

Radiation is typically administered in a dose range serving to "sterilize" cancer cells, rather than kill them in the metabolic sense, where "sterilized" means "no longer indefinitely clonogenic," i.e., able to give rise to an arbitrarily large number of descendents. The clonogenic assay is the standard means of assessing sensitivity to radiation. To carry out

this assay, cells in culture are irradiated with dose $D$ (in Grays) of ionizing radiation, then seeded on a Petri dish, well spaced from one another. After a duration of time spanning several doubling times, the percentage of cells that went on to produce colonies of 50 or more cells is tallied. After correcting for the plating efficiency, this percentage is referred to as the "surviving fraction" ($SF$) at dose $D$. This procedure is carried out for several values of $D$ in the range of interest and $SF$ versus $D$ plotted. One often then wishes to fit a mathematical equation to these data, identifying $SF$ as a function of $D$, for use in basic research or in planning a course of treatment with fractionated radiotherapy. For over fifty years, the most commonly assumed functional form has been $SF(D) = e^{-\alpha D - \beta D^2}$. This is known as the "linear-quadratic" (LQ) model. The LQ model has been moderately successful at fitting the clonogenic assay data, but there are data sets for which it fails utterly. See [1] for a discussion of its limitations, which paper also proposes an alternative known as the power model.

Another set of alternatives is provided by the target models. These assume that a cell contains one or more targets, which must be struck by a photon or particle of radiation one or more times, for the cell to be sterilized. The new model introduced in this paper is of this class. It is a single-target model and I assume that this target is just the genome. However, for the purpose of formulating a dose-survival equation, it is better to think of the target as the entrypoint, upon the cell membrane, of those photons/particles which are ultimately destined for the genome. That is, in the model, the target is the portion of cell membrane occluding the genome. This distinction minimizes confusion later in the paper, where I show that the number of strikes the target must receive for the cell to be sterilized varies with the underlying cellular state, probably due to differences in the resilience of the nuclear envelope.

## 2. Methods

An equation, motivated by first principles, was formulated for the surviving fraction ($SF$) as a function of dose in Grays ($D$). It was then hand-fit to several clonogenic assay data sets drawn from the published literature and the results plotted, using MATLAB R2020b (The MathWorks, Inc., Natick, MA, 2020).

## 3. Results

In terms of probability theory, the surviving fraction at dose $D$ is an estimator of the probability that a cancer cell will *not* be sterilized by this dose of radiation. Our task is thus to determine how this probability varies with $D$. When a cell is irradiated, three questions arise: (1) How many photons/particles of radiation will be emitted by the source in the direction of the cell's genome? In other words, how many photons/particles will strike the target on the cell membrane? (2) Given that $i$ photons/particles strike the target, will they cause genome damage? This requires them to penetrate the nuclear envelope. Let us denote by $u$ the probability that genome damage follows $i$ strikes upon the target. (3) Given that the target has received $i$ strikes and genome damage has occurred, will the cell be sterilized? Repair could take place or the damage, even if unrepaired, might not be such as to cause reproductive death. Let us denote by $p_0$ the probability that sterilization follows genome damage. With these definitions, we have the following formula for $SF$:

$$SF = 1 - up_0 \tag{1}$$

The values of $u$ and $p_0$ in this equation depend upon the exact number of strikes the target receives, the type of radiation being used, and the cancer cell itself.

*3.1. One strike versus two*

Suppose that, for a given population of cancer cells, $u$ and $p_0$ in Eq. 1 do not change with the number of strikes the target receives, provided it receives at least one. The probability of the target receiving at least one strike is $u_1 = 1 - e^{-kD}$, where $D$ is the dose in Grays and $k$ is a positive parameter varying directly with the cross-sectional area of the target (equivalently, the genome) and inversely with the per-photon or per-particle energy of the radiation being used. This formula follows from the fact that the number of strikes the target receives is Poisson-distributed with mean $kD$; see *Supplementary Information* for details. $SF(D)$ for these cells would then be:

$$\begin{aligned} SF &= (1 - u_1(D)) \cdot 1 + u_1(D)(1 - up_0) \\ &= 1 - u_1(D)up_0 \\ &= 1 - (1 - e^{-kD})up_0 \\ &= 1 - (1 - e^{-kD})P_0 \end{aligned} \tag{2}$$

The first line of Eq. 2 follows from the fact that, if the target receives zero strikes of radiation, each cancer cell will go on to produce a colony exceeding 50 cells with probability 1. If one or more strikes are received, sterilization will follow with probability $up_0$, which product I absorb into the single parameter $P_0$. A value of $P_0 < 1$ indicates radioresistivity.

Now consider a second population of cancer cells. These cells are never sterilized by a single strike of radiation ($up_0 = P_0 = 0$ for $i = 1$), but if the target receives two or more strikes ($i \geq 2$), then $u$ and $p_0$ in Eq. 1 are nonzero and constant. The probability that the target will receive at least two strikes is $u_2 = 1 - e^{-kD} - kDe^{-kD}$. Hence, $SF(D)$ for these cells would be:

$$\begin{aligned} SF &= (1 - u_2(D)) \cdot 1 + u_2(D)(1 - up_0) \\ &= 1 - u_2(D)up_0 \\ &= 1 - (1 - e^{-kD} - kDe^{-kD})up_0 \\ &= 1 - (1 - e^{-kD} - kDe^{-kD})P_0 \end{aligned} \tag{3}$$

Fig. 1 demonstrates how the shape of the survival curve $SF(D)$ changes depending upon the number of strikes the target must receive for sterilization to occur.

*3.2. State R versus state Q hypothesis*

Fig. 1 suggests, as a first pass, the following general formula for fitting clonogenic assay data collected using low-LET radiation, for non-radioresistant cells:

$$SF = p_1 e^{-kD} + p_2(e^{-kD} + kDe^{-kD}) \tag{4}$$

where $0 \leq p_1 \leq 1$, $0 \leq p_2 \leq 1$, and $p_1 + p_2 = 1$. $p_1$ (equivalently, $p_2$) modulates where $SF(D)$ falls on the spectrum between the two idealized cases shown at the bottom

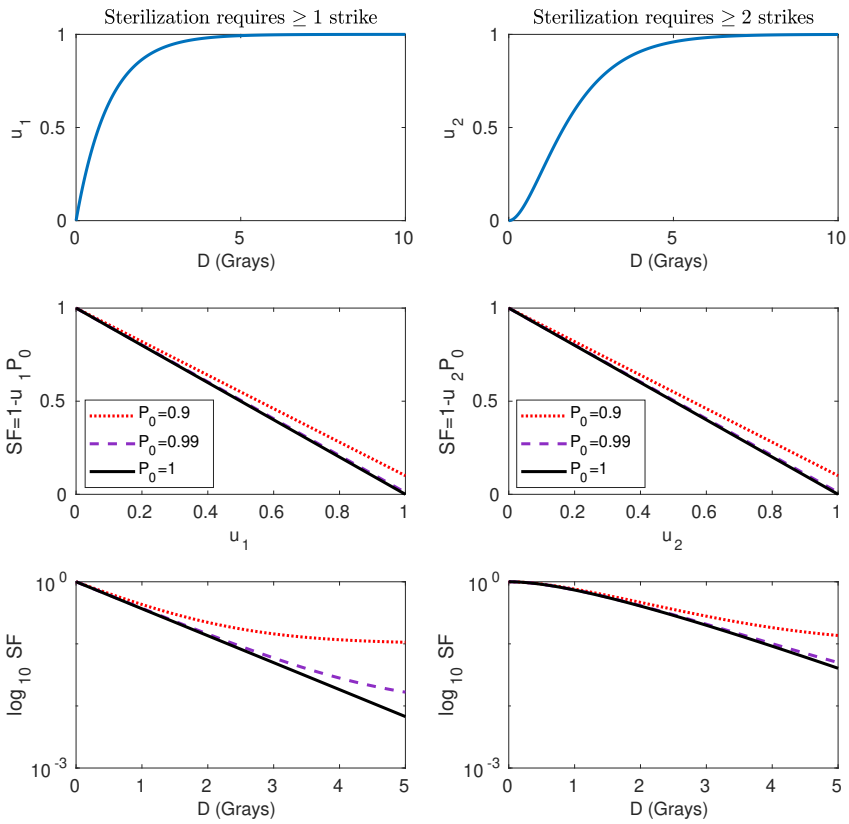


Figure 1: **The two typical survival curve shapes obtained using low-LET radiation.** *Upper left panel*: $u_1$ (probability of the target region of the cell membrane receiving one or more strikes of radiation) as a function of $D$. As $D$ increases from zero toward infinity, $u_1$ increases from 0 toward 1 in a way that is determined by Poisson statistics. *Middle left panel*: $SF$ as a function of $u_1$. In the absence of radioresistivity ($P_0 = 1$), $SF$ approaches 0 as $u_1$ approaches 1. When radioresistivity is present, $SF$ approaches $1 - P_0 \neq 0$. *Lower left panel*: $SF$ as a function of $D$. In the absence of radioresistivity, $SF(D)$ plotted on a semilog scale has the shape typically observed for rapidly proliferating cancer cells. When radioresistivity is present, $SF(D)$ is concave up. *Upper right panel*: $u_2$ (probability of the target region of the cell membrane receiving two or more strikes of radiation) as a function of $D$. Cf. upper left panel. *Middle right panel*: $SF$ as a function of $u_2$ (identical to middle left panel). *Lower right panel*: The different shape of $u_2(D)$ compared with $u_1(D)$ results in a different shape for $SF(D)$. In the absence of radioresistivity, $SF(D)$ plotted on a semilog scale has the shape typically observed for slowly proliferating cancer cells and normal cells. The shoulder region reflects the fact that two or more strikes have little probability of occurring until $D$ is sufficiently large. The shoulder region would have been more prominent had three (or more) strikes been required for sterilization. When radioresistivity is present, $SF(D)$ eventually becomes concave up as $D$ increases. In this figure, $k = 1$.

of Fig. 1. Why would clonogenic assay data obey this equation? I suggest the following hypothesis: the cell cycle and $G_0$ (i.e., all cellular activities) can be partitioned into two phenomenological states. In one of them, a cell will be sterilized if the target region of its cell membrane receives even one strike of low-LET radiation. In the other, the target must receive two strikes for sterilization to occur. $p_1$ and $p_2$ in Eq. 4 would then be the fraction of assayed cells in state one and state two, respectively, when radiation is administered. This is actually a bit of a simplification. Although two strikes, while a cell is in state two, guarantee sterilization for some cell lines (for example, the ovarian cells in Fig. 3), other cell lines are more resilient. The new dose-survival equation, introduced in the next section, takes this into account. State Q is best defined as the complement of state R, the latter being that cellular state in which one strike of low-LET radiation suffices for sterilization.

Decades of clonogenic assay data collected using low-LET radiation indicate that rapidly replicating cancer cells usually have $p_1$ large (straighter dose-survival curve) while slowly replicating cancer cells and normal, quiescent cells usually have $p_2$ large (curvier dose-survival curve). In nod to this, I henceforth refer to state one and state two as "state R" and "state Q," respectively. At the moment of irradiation, $p_R$ of assayed cells will be in state R and $p_Q = 1 - p_R$ will be in state Q. Since replication can be expected to be maximally asynchronous, $p_R$ and $p_Q$ are just the proportions of time cells spend in state R and state Q, respectively:

$$p_R = \frac{T_R}{T_R + T_Q} \tag{5}$$

$$p_Q = \frac{T_Q}{T_R + T_Q} \tag{6}$$

where $T_R$ is the time-duration of state R, $T_Q$ is the time-duration of state Q, and $T_D = T_R + T_Q$ is the doubling time.

The most parsimonious explanation for the observation that $p_Q$ increases as replication rate decreases (doubling time increases) is that the duration of state R varies little across different cancer cell lines, so that most of the variation in doubling time (17-80 hours [2]) is due to variation in $T_Q$. This motivates a hypothesis, presented later in the paper, for what states R and Q actually represent. For the moment, I emphasize that state R and state Q are not phases of the cell cycle. Rather, each phase of the cell cycle, and $G_0$, can be partitioned into two sets of activities, those corresponding to state R and those corresponding to state Q. The more radiation-resistant phases, and $G_0$, partake of state Q to a greater extent (i.e., $p_Q$, restricted to that phase, is larger). See Fig. 2 for a schematic of the state-R/state-Q hypothesis.

### *3.3. New dose-survival equation*

#### *3.3.1. Preliminary remarks regarding dosing*

The new dose-survival equation is derived under the assumption that radiation is administered over a fixed time interval $\Delta t$, with larger doses being achieved by using a higher dose rate. More typically, the dose rate is held constant and larger doses are achieved by increasing $\Delta t$. This may cause the new equation to overestimate $SF$ at low doses, as discussed at the end of this section.

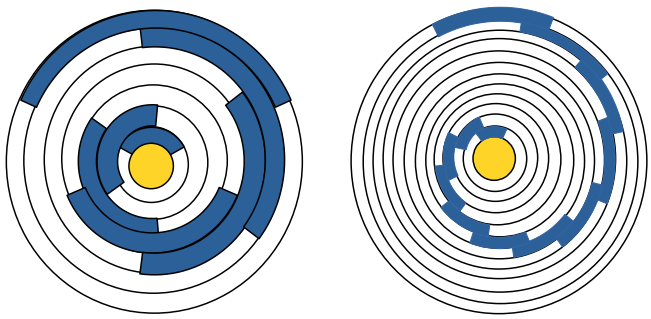

Figure 2: **"State Q versus state R" hypothesis.** A cancer cell's progression through the cell cycle can be depicted using a "cancer wheel" diagram. Imagine the cancer wheel as a clock-face with a hand (not shown) which moves clockwise, completing one revolution every $T_D$ hours (the doubling time). Each cancer cell, or set of synchronized cells, occupies one track of the cancer wheel. As the hand moves, cells move with it, progressing through the cell cycle. The dose-survival relationship that has been observed for low-LET radiation can be explained by the hypothesis that the cell cycle can be partitioned into two states, which differ in their sensitivity to low-LET radiation. I call these state R and state Q. When a cell is in state R (blue part of track), it will be sterilized if the target region of the cell membrane receives even one strike of low-LET radiation. When a cell is in state Q (white part of track), more than one strike is needed for this. If a population of cancer cells features maximally asynchronous replication, then all tracks will be equally occupied at the time of irradiation. Hence, $p_Q = \frac{T_Q}{T_Q+T_R}$ of the cells will be on a white region of track and $p_R = 1 - p_Q$ of the cells will be on a blue region, where $T_R$ is the time-duration of state R, $T_Q$ is the time-duration of state Q, and $T_D = T_Q + T_R$. If $T_R$ is more highly conserved than $T_Q$ across different cell lines, a trend will be observed wherein $p_Q$ is larger for cells with longer doubling times (more slowly replicating cells), causing them to have a curvier dose-survival relationship. *Left panel*: A hypothetical cancer cell line with doubling time 54 hours. 18 of these are spent in state R and 36 are spent in state Q. Hence, $p_R = 1/3$ and $p_Q = 2/3$. *Right panel*: A hypothetical cancer cell line with doubling time 180 hours; this is an extreme example, intended to illustrate the concept. The duration of state R is still 18 hours but the duration of state Q is now 162 hours. Hence, $p_R = 0.1$ and $p_Q = 0.9$. In this diagram, each cancer wheel track is partitioned into a single white region and a single blue region. This was done for simplicity. The true pattern is more fragmented, since each phase of the cell cycle is comprised of blue and white regions.

*3.3.2. Equations*

Eq. 4 is a special case of the more general Eq. 7 given below, where $p_1$ and $p_2$ are now called $p_R$ and $p_Q$, respectively. The key idea behind Eq. 7 is to condition $SF$ upon two things: (1) whether a cell is in state R or state Q when radiation is administered and (2) the exact number of strikes the target (i.e., target region of the cell membrane) receives. For each possible choice of (1) and (2) there is a copy of Eq. 1 and a pair of values for $u$ and $p_0$; since these cannot be identified separately from standard clonogenic assay data, they are absorbed into one constant. These are the $P_{0R,i}$ ($i \geq 1$) and $P_{0Q,i}$ ($i \geq 1$), which specify the probability of sterilization following $i$ strikes upon the target, given that a cell is in state R or state Q. Each sterilization probability reflects all of the possible ways in which $i$ strikes could arrive within $\Delta t$, the dosing time window. Note that $P_{0R,1} \leq P_{0R,2} \leq P_{0R,3} \leq \cdots$ and $P_{0Q,1} \leq P_{0Q,2} \leq P_{0Q,3} \leq \cdots$, since as the number of strikes increases, the probability that sterilization will follow cannot decrease.

$$\begin{aligned} SF(D) = p_R\ e^{-kD}[1 + kD(1 - P_{0R,1}) + \cdots + \frac{1}{i!}(kD)^i(1 - P_{0R,i}) + \ldots] \\ + p_Q\ e^{-kD}[1 + kD(1 - P_{0Q,1}) + \cdots + \frac{1}{i!}(kD)^i(1 - P_{0Q,i}) + \ldots] \end{aligned} \tag{7}$$

For most cancer cells, we can use Eq. 8 given below. This is a special case of Eq. 7 where we have set $P_{0R,i} = 1$ for all $i \geq 1$, $P_{0Q,1} = 0$, and $P_{0Q,i} = 1$ for all $i \geq 3$. $P_{0Q,2}$ is a free parameter ranging between 0 and 1. In English this means: if a cell is in state R, then one or more strikes upon the target guarantees sterilization. If a cell is in state Q, then one strike upon the target has no chance of achieving sterilization, two strikes have some chance, and three or more strikes guarantee it.

$$SF(D) = p_R e^{-kD} + p_Q e^{-kD}(1 + kD + (1 - P_{0Q,2})\frac{1}{2}k^2D^2) \tag{8}$$

When a good fit to the clonogenic assay data cannot be obtained using Eq. 8, the cells are either ultrasensitive or radioresistant. Ultrasensitive mutants have $P_{0Q,1} > 0$ and $P_{0Q,2} = 1$, requiring a slight modification of Eq. 8 (not shown, but obvious). Radioresistant cells have either $P_{0R,1} < 1$ (one strike upon the target does not guarantee sterilization, in state R) and/or $P_{0Q,3} < 1$ (three strikes upon the target do not guarantee sterilization, in state Q). Eq. 7 must be used for them. When cells are radioresistant because $P_{0Q,3} < 1$, it is possible that $P_{0Q,3} = 1$ for a higher dose rate; this is discussed later. Regarding high-LET radiation, the clonogenic assay data indicate that one strike of this type of radiation guarantees sterilization, whether a cell is in state Q or state R. Since state Q behaves identically to state R, we have simply $SF = e^{-kD}$. However, note that the size of the target region of the cell membrane might be larger for high-LET radiation than for low-LET radiation, resulting in a larger value of $k$, even if the per-photon or per-particle energy were the same. This would occur if such radiation could miss the genome by a wider margin and still be effective.

*3.3.3. Terminal linear region*

No matter how radioresistant a cell line is, there will be a minimal number $N$ such that, if the target receives $N$ or more strikes, sterilization is guaranteed. This stems from the

fact that no real cell line can withstand an arbitrarily large number of strikes within a finite time interval, unlike the idealized radioresistant cancer cells ($P_0 < 1$) of Fig. 1. In terms of Eq. 7, as $i$ increases, the $P_{0R,i}$ and $P_{0Q,i}$ eventually attain 1, causing all but a finite number of terms to zero out. Since the critical number of strikes generally differs for state R and state Q, let us call the larger of these $N$. A cell whose target receives $N$ or more strikes will be sterilized, regardless of its state. For highly radioresistant cells, like the rr HTB140 cells in Fig. 3, $N$ can be quite high; it is 11 for them. A large value of $N$ causes the clonogenic assay data to feature a prominent "bustle" region. Beyond the bustle region, the graph of $ln(SF(D))$ approaches a linear decline with slope $-k$. The terminal linear region constitutes a "signature" assuring us that we have identified the entire graph and can confidently extrapolate out to high doses. If this region cannot be uniquely identified from the clonogenic assay data, then more data must be collected, at higher doses. Note that this region enables us to identify $k$ in isolation from other parameters.

*3.3.4. Considerations that apply when using a constant dose rate*

Eq. 7 assumes that a given number of strikes has a certain probability of causing sterilization, with no other influencing factor (other than whether the cell is in state R or state Q). This is a realistic assumption when radiation is administered over a constant time interval $\Delta t$, the different doses being achieved by varying the dose rate. More typically though, the dose rate is held constant and $\Delta t$ is varied. This poses a potential problem: the probability that sterilization will be achieved by a particular number of strikes should, in general, depend upon the time interval within which they arrive. In particular, it should increase as $\Delta t$ shrinks to zero and the energy of the strikes is deposited at nearly the same time. Since the $P_{0R,i}$ ($i \geq 2$) and $P_{0Q,i}$ ($i \geq 2$) vary with $\Delta t$, and $\Delta t$ varies with $D$ (when dose rate is constant), the $P_{0R,i}$ ($i \geq 2$) and $P_{0Q,i}$ ($i \geq 2$) are functions of $D$, rather than constant, as Eq. 7 assumes. Despite this, Eq. 7 still fits the clonogenic assay data shown in Fig. 3. Each of these data sets was collected using a constant dose rate. A possible explanation for this success may be as follows.

The sterilization probabilities $P_{0R,i}$ ($i \geq 2$) and $P_{0Q,i}$ ($i \geq 2$) are nonincreasing functions of $\Delta t$ (equivalently, $D$): each should approach a maximal value as $\Delta t \to 0$ (equivalently, $D \to 0$), and as $\Delta t$ (equivalently, $D$) increases, each should decrease or hold steady, eventually plateauing to some constant value. In the plateau region, the time intervals between successive strikes are, with high probability, long enough for the "dust to settle" between strikes. That is, they are long enough that a (damaged) steady state is attained after each strike, so that the exact timing of the next strike is not relevant to the additional damage it effects. In the plateau region, only the number of strikes matter for the sterilization probabilities, not the time gaps between them. Since they are constant in $\Delta t$, they are constant in $D$ and Eq. 7 applies. This analysis assumes that the few minutes over which radiation is administered are not long enough for significant repair of sublethal damage to occur.

The above reasoning suggests that, for the data sets shown in Fig. 3, the time windows over which radiation was delivered were, in the main, long enough for the sterilization probabilities to be in their plateau regions. However, for some data sets, the fit is imperfect at $D = 2$. This indicates that, for this shortest dosing time window, at least one sterilization probability exceeded its plateau-region value. As a general rule, when a constant dose rate is used, the new dose-survival equation is more accurate at higher doses, for which $\Delta t$ is

longer; at smaller doses, it may overestimate $SF$. This problem is minimized when using a low dose rate, since the shortest $\Delta t$ will be longer.

Mathematically, it would be much better if radiation were delivered over a constant time interval and different doses were achieved by varying the dose rate. This would cause Eq. 7 to hold without reservation, improving our ability to fit clonogenic assay data and identify underlying cellular parameters. It would also enable us to use high dose rates without fear of Eq. 7 becoming inaccurate. Higher dose rates have the advantage of shrinking the bustle region, when one exists, since guaranteed sterilization can generally be achieved with fewer strikes (i.e., $N$ is smaller) if these arrive within a shorter time window. More generally, higher dose rates serve to increase the sterilization probabilities associated with $i \geq 2$ strikes. For this reason, the new convention for classifying cells as radioresistant has a proviso: certain cell lines might have $P_{0Q,3} < 1$ for one dose rate, but $P_{0Q,3} = 1$ for a higher one.

### *3.4. Sample fits*

Fig. 3 shows sample fits of the new dose-survival equation to several clonogenic assay data sets found in the published literature. The PC3, rr PC3, and HTB140 fits show the predicted imperfection at low doses. The AG6000 fit does not and is not predicted to, since $P_{0Q,2} = 1$: as the time window for dose delivery becomes smaller, a sterilization probability of 1 cannot become any larger. We also do not see the imperfection for the SWg cells. These cells would be classified as highly radioresistant by the convention of this paper. The sterilization probabilities $P_{0Q,2} = 0$ and $P_{0Q,3} = 0$ are compatible with a scenario in which $\Delta t$ (equivalently, $D$) must be very small indeed before $P_{0Q,3}$ and $P_{0Q,2}$ become nonzero. Hence, the imperfection might only appear at, say, $D = 0.5$ Gy.

Besides successfully fitting the clonogenic assay data, the new equation enables us to decompose $SF(D)$ into the theoretical state-R ($SF_R(D)$) and state-Q ($SF_Q(D)$) dose-survival curves. If $SF_Q(D)$ is used instead of $SF(D)$ to select a fraction size for fractionated radiotherapy, cell cycle phase is eliminated as a variable in treatment outcome. $SF_R(D)$ and $SF_Q(D)$ may also be useful for standardizing the results reported for clonogenic assays; see *Discussion*.

Let us use Eq. 5 to estimate the duration of state R from the AG6000 data. AG6000 cells are gemcitabine-resistant (i.e., resistant to its action as a chemotherapeutic agent) descendents of A2780 ovarian cancer cells and have an exponential-phase doubling time of 22-24 hours [3]. The data shown were collected for control cells in experiments investigating radiosensitization with gemcitabine. The authors state that the control cells were in the exponential phase of growth when radiation was administered [4]. Since the doubling time is known, we can estimate the duration of state R as $T_R = p_R T_D = 0.75 \cdot 24 = 18$ hours. In the *Discussion*, I offer a hypothesis regarding what states Q and R actually represent. This hypothesis implies that the duration of state R is highly conserved not only across different cell lines, but also within a cell line, as the conditions in cell culture vary. Hence, we can use this value of $T_R$ to estimate the actual doubling time of the PC3 cells, which were at 80% confluence [5] (i.e., far from the exponential phase) when radiation was administered. We obtain $T_{D,act} = \frac{T_R}{p_R} = \frac{18}{0.2} = 90$ hours. This is very reasonable, given that PC3 cells have a reported exponential-phase doubling time of 33-40 hours [6].

SWg cells are gemcitabine-resistant descendents of SW1573 lung cancer cells; the latter have an exponential-phase doubling time of 22-24 hours [3]. The data shown are for control

cells in the same experiments investigating radiosensitization with gemcitabine. Cells were reported to be in the exponential phase of growth when radiation was administered. However, the estimated doubling time for these cells is 90 hours, since $p_R = 0.2$. I suspect that the SWg cells experience contact inhibition, as has been reported for a different lung cancer cell line [7]. Although far from carrying capacity, their actual doubling time, at the moment of irradiation, was probably much longer than 24 hours. The fit shown in Fig. 3 has $p_R$ set to the largest value that is consistent with the data, corresponding to the shortest possible doubling time, under the assumption that state R is normally radiosensitive. If we allow state R to be radioresistant, $p_R$ can have any value. Regardless of what is going on with these cells, they are an exception to the general rule that rapidly replicating cells have a straight dose-survival relationship.

For cells that are extremely radioresistant (ones having a pronounced bustle region), multiple fits are possible. An example of this is provided by the rr HTB140 melanoma cells. The competing fits shown in Fig. 3 differ in how they apportion "responsibility" for the radioresistivity between states Q and R. This translates into different values of $p_Q$ and different theoretical state-R and state-Q dose-survival curves. How could we decide between the two fits? One approach would be to redo the clonogenic assay, this time for cells at nearly 100% confluency. $p_Q$ would be 1 or nearly 1, while $SF_Q(D)$ and $SF_R(D)$ (if the latter were identifiable) would be the same as in the correct fit, assuming the speculations in the *Discussion* are correct.

There is another possible criterion. The HTB140 cells had a doubling time of 24 hours at the time of irradiation [8]. Using this information, the fit shown in the second panel of the bottom row estimates the duration of state R as $T_R = 18$ hours ($T_Q = 6$ hours), in accord with the AG6000 data. The fit shown in the third panel of the bottom row estimates it as $T_R = 21.6$ hours ($T_Q = 2.4$ hours). $T_Q = 2.4$ hours seems unrealistically short (see *Discussion*), indicating that we should go with the fit in the second panel. To establish a rigorous time-duration criterion for deciding between competing fits, $T_R$ and $T_Q$ should be estimated for a large number of normally radiosensitive cells. This would identify (1) the typical range for $T_R$, which should be pretty narrow, and (2) any lower bound on $T_Q$. For this approach to work, we must know cellular doubling times with relative certainty, both for the "database" cell lines used to establish the criterion, and when the criterion is being applied. Toward this end, clonogenic assay data should be collected for cells undergoing exponential growth in medium that is replete with nutrients, so that the doubling time is known with greatest precision.

### *3.5. Derivation of the LQ model*

We can write Eq. 7 as follows:

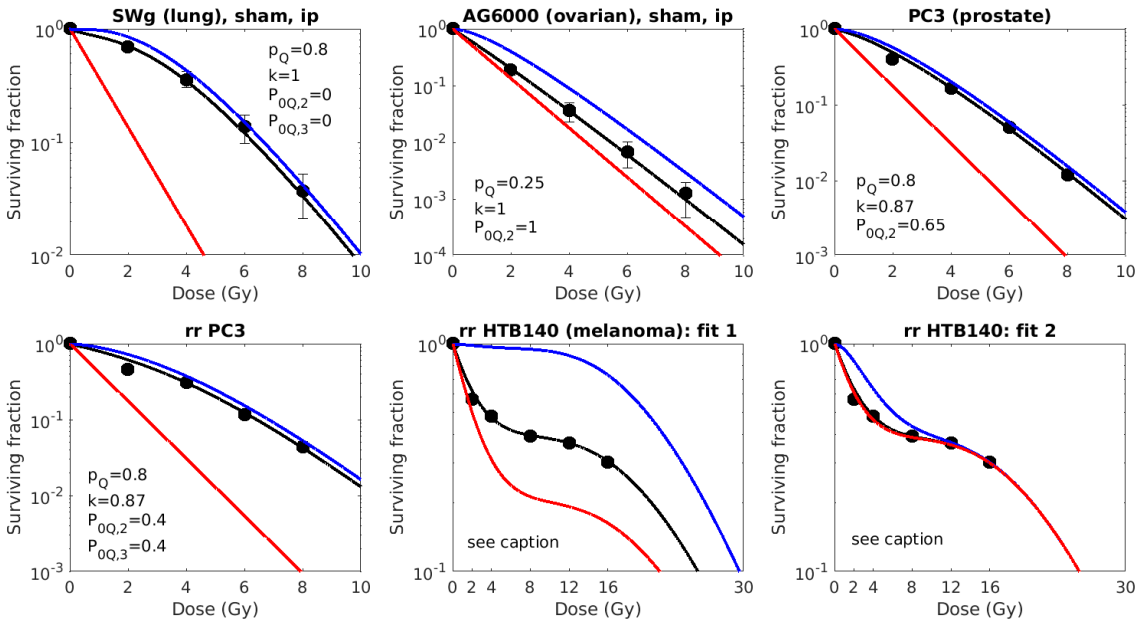


Figure 3: **Sample fits of the new dose-survival equation to previously published clonogenic assay data sets. Also shown is the theoretical state-R/state-Q decomposition.** *Data sets*: The SWg and AG6000 data sets are reproduced from Fig. 4 of [9]. These data were first published in [4], which paper investigates radiosensitization with gemcitabine. "Sham" indicates that cells were not treated with gemcitabine and "ip" means "immediately plated." The PC3 and radioresistant PC3 data sets are from Fig. 2 of [5]. Second and third panels of the bottom row present two alternative fits of Eq. 7 to a data set collected for HTB140, an ultra-radioresistant human melanoma cell line. These data are reproduced from [1] and were originally published in [8]. *Model fits*: Optimal fits are shown in black. The short form of the dose-survival equation (Eq. 8) provides a good fit for the AG6000 and PC3 cells. Hence, by the convention introduced in this paper, these two cell lines are normally radiosensitive. The other three cell lines require the full equation (Eq. 7) and are hence classified as radioresistant. Note that $p_Q$ reflects the actual doubling time of irradiated cells, which depends upon the conditions in cell culture. $P_{0Q,1} = 0$ for all cell lines since low-LET radiation is used; aside from this, where $P_{0Q,i}$ and $P_{0R,i}$ are not specified, they are equal to 1. *HTB140 cells*: The fit shown in the middle bottom panel has parameter values $p_Q = 0.25$, $k = 0.5$, $P_{0R,i} = 0.8$ $(1 \leq i \leq 10)$, $P_{0Q,1} = 0$, $P_{0Q,i} = 0.05$ $(2 \leq i \leq 9)$, $P_{0Q,10} = 0.5$. The fit shown in the right bottom panel has parameter values $p_Q = 0.1$, $k = 0.5$, $P_{0R,i} = 0.62$ $(1 \leq i \leq 9)$, $P_{0R,10} = 0.72$, $P_{0Q,1} = 0$, $P_{0Q,i} = 0.62$ $(2 \leq i \leq 9)$, $P_{0Q,10} = 0.72$. *Theoretical decomposition*: The theoretical state-R and state-Q dose-survival curves are shown in red and blue, respectively. If $SF_Q(D)$, rather than $SF(D)$, is used to select a fraction size for fractionated radiotherapy, cell cycle phase is eliminated as a variable in treatment outcome. Different cell culture conditions will yield different values for $p_Q$, but $SF_R(D)$ and $SF_Q(D)$ are probably invariant.

$$
\begin{aligned}
SF(D) &= e^{ln(SF(D))} && (9)\\
&= e^{ln(e^{-kD}[p_R(1+(1-P_{0R,1})kD+\cdots)+p_Q(1+(1-P_{0Q,1})kD+\cdots)])} && (10)\\
&= e^{-kD+ln[1+(p_R(1-P_{0R,1})+p_Q(1-P_{0Q,1}))kD+\frac{(p_R(1-P_{0R,2})+p_Q(1-P_{0Q,2}))}{2}k^2D^2+\cdots]} && (11)\\
&= e^{-kD+ln(1+aD+bD^2+\cdots)} && (12)\\
&= e^{-kD+(aD+bD^2+\cdots)-\frac{(aD+bD^2+\cdots)^2}{2}+h.o.} && (13)\\
&= e^{(a-k)D+(b-\frac{1}{2}a^2)D^2+h.o.} && (14)\\
&\sim e^{-\alpha D-\beta D^2} && (15)
\end{aligned}
$$

The first line is an identity. In the second line, I insert Eq. 7 for $SF(D)$. Note that, for any real cell line, only a finite number of terms are represented by the dots; that is, Eq. 7 is in closed form. In the third line, I use the fact that $ln(xy) = ln(x) + ln(y)$, and also rewrite the bracketed expression in a way which makes clear that it is of the form given in line four. In line five, I rewrite line four using the Taylor expansion of $ln(1 + x)$ about $x = 0$. This gives rise to a power series in $D$. In line six, I collect its like powers. The LQ model (line seven) is obtained by truncating the power series at second order. $\alpha$ and $\beta$ are given by:

$$
\begin{aligned}
\alpha &= k - a\\
\beta &= \frac{a^2}{2} - b
\end{aligned} \tag{16}
$$

where

$$
\begin{aligned}
a &= k(1 - (p_R P_{0R,1} + p_Q P_{0Q,1}))\\
b &= \frac{k^2}{2}(1 - (p_R P_{0R,2} + p_Q P_{0Q,2}))
\end{aligned} \tag{17}
$$

In the *Supplementary Information*, I provide formulas for $\alpha$ and $\beta$ in terms of the new model's parameters for the case where Eq. 8 fits the clonogenic assay data well, and show how $\alpha/\beta$ varies with replication rate. Incidentally, the only time the LQ model is exact is when $SF = e^{-kD}$. In all other cases, the power series in the exponent of $e$ has an infinite number of nonzero terms. For example, if $p_Q = 1$, $P_{0Q,1} = 0$, and $P_{0Q,2} = 1$, then $SF = e^{-kD}(1+kD)$ and $ln(SF) = -\frac{1}{2}k^2D^2 + \frac{1}{3}k^3D^3 + h.o.$

The LQ model is actually just the second member of an infinite family of models, beginning with $SF(D) = e^{-\alpha D}$, the linear model. After the LQ model comes the LQC model, of form $SF(D) = e^{-\alpha D-\beta D^2-\gamma D^3}$. The higher-order members of this family provide an appreciably better fit at high doses, and an appreciably better fit at all doses when radioresistivity is present and $\alpha$ and $\beta$ are near zero.

## 4. Discussion

Let us now consider the question of which cellular activities correspond to state R and which to state Q. I speculate that state-R activities are ones for which the rate of ATP hydrolysis within the nucleus is high, and that there is an ANT transporter, analogous to

the one located in the inner mitochondrial membrane, embedded in the nuclear envelope. This antiporter acts like a revolving door, exporting one intranuclear ADP to cytoplasm for every cytoplasmic ATP it imports. Such a transporter would be extremely useful, as it would prevent hydrolysis reactions taking place within the nucleus from becoming thermodynamically inhibited as ADP accumulates. Assuming it exists, it would be very active when the rate of ATP hydrolysis within the nucleus is high. This would put a mechanical strain on the nuclear envelope, enabling one particle or photon of low-LET radiation, after entering the cell membrane at the target location, to break the nuclear envelope and still have enough energy left over to inflict damage upon the genome which is irreparable and which renders the cell reproductively unviable. Hence, $P_{0R,1} = 1$. Stages of mitosis where no nuclear envelope is present are also subsumed under state R.

State Q is simply the complement of state R, comprised of all cellular activities during which there is not so great a demand for ATP hydrolysis within the nucleus. This probably includes (1) times when the cell is amassing the raw materials (amino acids and lipids) needed to duplicate itself and (2) most of $G_0$, although note that quiescent cells may still have occasional need for rapid ATP hydrolysis within the nucleus. The reduced mechanical strain on the nuclear envelope during state Q would render it more resilient, so that if a single strike of low-LET radiation were to rupture it, little energy would be left over to damage the enclosed genome, and any damage could be successfully repaired. Hence, $P_{0Q,1} = 0$. Two strikes are successful with probability $0 \leq P_{0Q,2} \leq 1$.

If this hypothesis is correct, it is understandable why state R's duration would be more highly conserved than state Q's across different cell lines: a high rate of ATP hydrolysis within the nucleus can only be sustained so long as tasks are being carried out there which require it. These tasks are probably highly conserved across different cell lines and when they are completed, the intense demand for ATP within the nucleus ends. In contrast, amassing the necessary raw materials is an activity which should take an amount of time that is highly variable, if only because different cells have different ratios of surface area to volume. I suspect that this activity accounts for most of the variability, across different cell lines, in the duration of state Q.

This hypothesis also provides insight into how clonogenic assay data vary with the conditions in irradiated cell culture (type of medium used, percent confluence). As nutrients and/or space become limited, the actual doubling time ($T_{D,act}$) should increase, but the duration of state R ($T_R$) should remain constant, assuming that glucose is not so depleted as to limit the rate of ATP synthesis. $SF_R(D)$ should be invariant. $T_Q = T_{D,act} - T_R$ should increase. $SF_Q(D)$ should be invariant if there is no ATP hydrolysis within the nucleus during state Q. If there is, then it is conceivable that $SF_Q(D)$ becomes more "shouldery" as resources become limited, but I suspect that $SF_Q(D)$ is invariant even in this case. This question could be settled by fitting the new dose-survival equation to a number of clonogenic assay data sets, collected for the same cell line under different culture conditions, and seeing whether $SF_R(D)$ and $SF_Q(D)$ are the same.

If they are invariant, then $SF_R(D)$ and $SF_Q(D)$ constitute "basis functions" for a given cell line: as conditions in cell culture vary, the value of $p_R$ changes, shifting where $SF(D)$ falls on the spectrum between $SF_R(D)$ and $SF_Q(D)$. $p_R = \frac{T_R}{T_D}$ would be maximal when medium is replete with nutrients and cells are in exponential growth, producing the straightest possible dose-survival relationship for the cell line. In less enriched medium, or at high confluence,

the actual doubling time would be much longer than $T_D$, causing $p_R = \frac{T_R}{T_{D,act}}$ to be smaller, and producing a curvier dose-survival relationship. Reporting $SF_R(D)$ and $SF_Q(D)$ for a given cell line would then enable us to describe clonogenic assay results in a way which does not depend upon the conditions in irradiated cell culture.

### *4.1. OER phenomenon*

The above line of thought suggests a novel hypothesis for the "oxygen enhancement ratio" (OER) phenomenon. Cancer cells are known for having a much smaller flux through oxidative phosphorylation than normal cells, relying heavily on glycolysis to produce ATP and shunting most of the pyruvate that is produced through lactate dehydrogenase. However, glycolysis itself yields only 2 ATP per glucose, while oxidative phophorylation yields another 30 ATP per glucose [10]. Since the yield is so much higher for oxidative phosphorylation, even if pyruvate enters the Krebs cycle at (for example) 10% the normal rate, oxidative phosophorylation would still be responsible for more than half of the ATP appearing in cytoplasm. The loss of this during hypoxia would cut in half the maximal flux through a nuclear ANT transporter. This would alleviate mechanical strain on the nuclear envelope during state R, decreasing $P_{0R,1}$ and rendering state R more resistant to low-LET radiation. The same would occur for state Q, if there were a sufficiently high demand for ATP hydrolysis within the nucleus during this state.

It would be informative to treat cells, prior to irradiation, with a (reversible) inhibitor of oxidative phosphorylation and see what effect this has on the dose-survival relationship. If the above hypothesis is correct, $SF(D)$ will at least become more radiation-resistant. At most, $SF(D)$ will look as though it were collected during hypoxia. This experiment would decide the extent to which the OER phenomenon is really an OXPHOS phenomenon, mediated by an ANT transporter embedded in the nuclear envelope. A similar hypothesis applies for facultative anaerobes, such as *E. coli*. For them, the role of the nuclear envelope is played by the outer and inner plasma membranes: when oxygen is present and there is a demand for ATP hydrolysis within the bacterium, the F1F0-ATPase embedded in the inner membrane is very active, putting a mechanical strain on the membrane and rendering it more vulnerable to rupture by ionizing radiation.

Even if OXPHOS does not account for most of the OER phenomenon, the latter might still be mediated by the nuclear envelope, rather than by processes downstream of its rupture. Water in the perinuclear space forms hydrogen bonds with head groups on the inner and outer nuclear membranes, stabilizing the nuclear envelope. Liquid water is comprised of water clusters, which are cliques of hydrogen-bonded $H_2O$ molecules. Yuan et al. recently reported that $O_2$ molecules cannot stably exist within water clusters and instead reside at their interfaces [11]. Importantly, they found that as the concentration of dissolved oxygen increases, the average number of water molecules within a cluster decreases. If water cluster size is larger than normal during hypoxia, this may enhance the stability of the nuclear envelope, rendering it more resilient to low-LET radiation. The same effect would occur in the periplasm of bacteria, where water forms hydrogen bonds with head groups on the inner and outer plasma membranes.

## 5. Supplementary Information

### *5.1. Meaning of the parameter k*

Consider a cancer cell, located a distance $r$ away from a source of ionizing radiation, with no intervening material. Denote as the "target" the region of its cell membrane occluding the genome. Denote by $A_{CS,targ}$ the cross-sectional area presented by the target (equivalently, the genome) to the radiation source. Denote by $A$ the source activity, in mean number of decays per second. Let us administer a single dose of radiation to this cancer cell, by unshielding the source for $\Delta t$ seconds. The number of photons or particles of radiation striking the target during this time interval is Poisson-distributed with mean $\lambda$, where

$$\lambda = A\ \Delta t\ \frac{A_{CS,targ}}{4\pi r^2} \tag{18}$$

Denote by $A_{CS,cell}$ the cross-sectional area presented by the cell (in its entirety) to the radiation source. The number of photons or particles of radiation striking the cell (anywhere, not just at the target) is Poisson-distributed with mean $\lambda_{cell}$, where

$$\lambda_{cell} = A\ \Delta t\ \frac{A_{CS,cell}}{4\pi r^2} \tag{19}$$

By definition, the cancer cell receives the following dose in Grays (Joules absorbed energy per kilogram matter):

$$D = \frac{\lambda_{cell} \cdot E}{M_{cell}} \tag{20}$$

where $E$ is the energy, in Joules, per photon/particle of the type of radiation being used (X-rays, neutrons, $\alpha$-particles, etc.) and $M_{cell}$ is the mass of the cell in kilograms. Note that the actual amount of energy absorbed by the cell is a stochastic random variable, since the number of emitted photons/particles is a stochastic random variable. The dose in Grays is thus the expected value (mean) of the absorbed energy, per kilogram cell.

From Eqs. 19 and 20 we see that when a cancer cell is irradiated, the number of Grays it receives is directly proportional to the ratio $A_{CS,cell}/M_{cell}$. This will be different for cells of different sizes because the ratio of surface area (equal to four times the cross-sectional area) to volume is not constant for a sphere as its radius changes. For example, for the same experimental setup, a larger cell would receive a slightly smaller dose in Grays than would a smaller cell. Even for the same cell, $A_{CS,cell}/M_{cell}$ varies over the cell cycle, since the cell is growing. In light of this, it must be that when a dose is reported in Grays for a clonogenic assay, an average value has been assumed for the ratio $A_{CS,cell}/M_{cell}$. I call this factor $R_{area/mass}$. Combining Eqs. 19 and 20 and substituting $R_{area/mass}$ for $A_{CS,cell}/M_{cell}$, we obtain the following equation for the *reported* dose in Grays:

$$D = A\ \Delta t\ \frac{R_{area/mass} E}{4\pi r^2} \tag{21}$$

Combining Eqs. 21 and 18 yields:

$$\lambda = D\left(\frac{A_{CS,targ}}{R_{area/mass}E}\right) \tag{22}$$

$$= kD \tag{23}$$

Hence, $k$ is directly proportional to the cross-sectional area presented by the genome to the radiation source, and inversely proportional to the per-photon/per-particle energy of the type of radiation being used. We can now express, in terms of the *reported* dose in Grays, the probability that the target will receive various numbers of strikes of radiation. The probability that it will receive exactly $i$ strikes is:

$$P_i = \frac{(kD)^i e^{-kD}}{i!} \tag{24}$$

The probability that it will receive one or more strikes of radiation is:

$$u_1 = 1 - e^{-kD} \tag{25}$$

The probability that it will receive two or more strikes of radiation is:

$$u_2 = 1 - e^{-kD}(kD + 1) \tag{26}$$

Fig. 4 shows that as $k$ increases, the graph of $SF$ versus $D$ becomes compressed horizontally, so that smaller doses correspond to a smaller surviving fraction.

*5.2. $\alpha/\beta$ and replication rate*

For clonogenic assay data sets that are well fit by Eq. 8, we obtain the following formulae for $\alpha$ and $\beta$ in terms of the new model's parameters:

$$\alpha = p_R k$$
$$\beta = \frac{k^2}{2}(p_R - 1)(p_R - P_{0Q,2}) \tag{27}$$

Viewed as functions of $p_R$, $\alpha$ is a line and $\beta$ is a parabola. Although $\alpha$ is always non-negative, it is possible for $\beta$ to be negative. This occurs, for a given $p_R$, if state Q is resilient enough that $P_{0Q,2} < p_R$. This is consistent with intuition: if state Q were infinitely "shouldery," i.e., totally immune to radiation, so that all state-Q sterilization probabilities (including $P_{0Q,2}$) were zero, $SF(D)$ would simply be $SF(D) = p_Q + p_R e^{-kD}$. The graph of this function is concave up and hence its LQ approximation has $\beta < 0$.

Let us examine how $\alpha$ and $\beta$ change as $p_R$ decreases. As $p_R \to 0$, $\alpha \to 0$ and $\beta \to \frac{k^2}{2}P_{0Q,2}$. Thus, slowly replicating cells have $\alpha/\beta$ small and positive, provided $P_{0Q,2} \neq 0$. If $P_{0Q,2} = 0$, $\alpha/\beta \to -\frac{2}{k}$ as $p_R \to 0$. Assuming the values of $k$ in Fig. 3 are typical, this limit is of order 1. However, this case is not really seen in practice: since both $\alpha$ and $\beta$ are approaching zero, the LQ model would approach simply $SF = 1$, indicating the need for the higher-order terms in $ln(SF(D))$. When confronted with such a data set and armed only with the LQ model, the best-fit values for $\alpha$ and $\beta$ would not be the actual power-series coefficients. For example, the best LQ approximation to Eq. 8 with $k = 1$, $P_{0Q,2} = 0$, and $p_R = 0$, for $0 \leq D \leq 10$, has $\alpha = 0.13$ and $\beta = 0.05$; since these are not the asymptotically correct coefficients, the

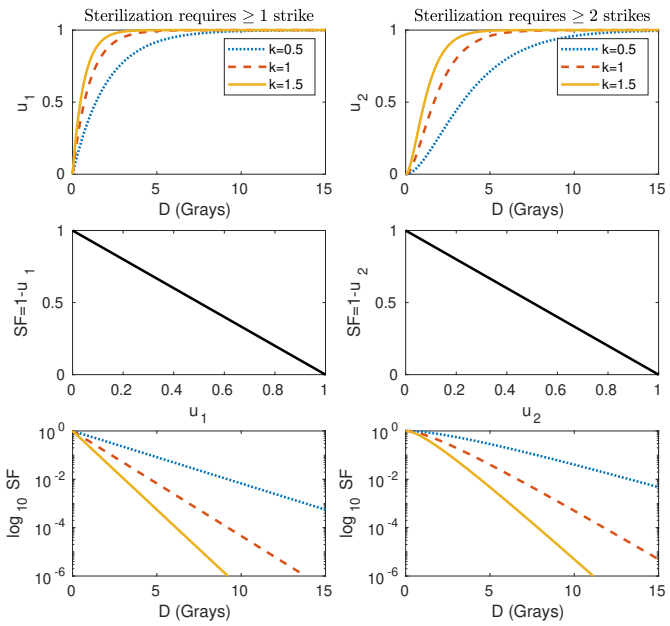


Figure 4: **Effect of varying $k$ on the graph of $SF$ versus $D$.** Compare with Fig. 1. *Upper panels*: The number of strikes received by the target on the cell membrane is Poisson-distributed with mean $kD$. As $D \to \infty$, $u_1$ (the probability of the target receiving at least one strike) and $u_2$ (the probability of the target receiving at least two strikes) approach 1. They do this more rapidly for larger values of $k$. *Middle panels*: These are shown for comparison with Fig. 1. *Bottom panels*: The more rapid approach of $u_1$ and $u_2$ to 1 as $k$ increases translates into a horizontal compression of the graph of $SF$ versus $D$.

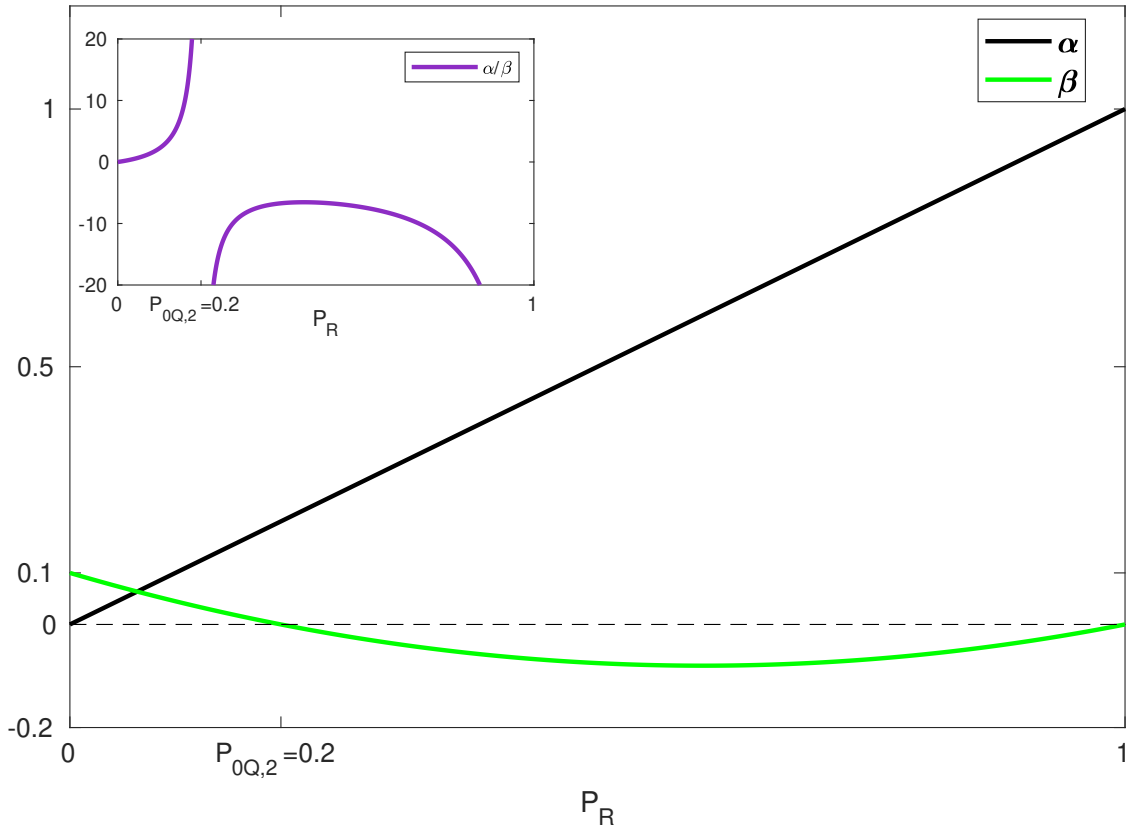


Figure 5: **$\alpha$, $\beta$, and $\alpha/\beta$ as functions of $p_R$, the proportion of time cells spend in state R.** The LQ model can be obtained from Eq. 7 by taking a Taylor expansion. For cells whose clonogenic assay data are well fit by Eq. 8, $\alpha$ and $\beta$ are as given in Eq. 27. As $p_R \to 0$ (replication rate slows), $\alpha/\beta$ approaches zero from the positive direction. As $p_R \to 1$ (replication rate increases), $|\alpha/\beta|$ approaches infinity. The range of $p_R$ over which $\alpha/\beta$ is positive is $(0, P_{0Q,2})$. In this figure, $k = 1$ and $P_{0Q,2} = 0.2$.

LQ model does *not* become better and better, for this case, as $D \to 0$ (not shown). This issue exists for any "sufficiently shouldery" data set, such as might be collected for normal, quiescent cells with a particularly resilient state Q.

Let us now see what happens as $p_R$ increases. As $p_R \to P_{0Q,2}$ (the first root of $\beta(p_R)$), $\beta$ approaches zero from the positive direction, causing $\alpha/\beta$ to approach positive infinity. On the other side of this root, $\alpha/\beta$ changes sign. As $p_R$ continues to increase, $\alpha/\beta$ comes in from negative infinity (becomes smaller in magnitude), then shoots out again toward negative infinity as $p_R$ closes in on 1. This assumes that $0 < P_{0Q,2} < 1$. When $P_{0Q,2} = 1$, the analysis is more straightforward: as $p_R$ increases from 0 to 1, $\alpha/\beta$ increases from zero to positive infinity. When $P_{0Q,2} = 0$, then as $p_R$ increases from 0 to 1, $\alpha/\beta$ decreases from $-\frac{2}{k}$ to negative infinity. Thus, we have the general rule that $\alpha/\beta$ is large in magnitude for rapidly replicating cells. Fig. 5 shows plots of $\alpha$, $\beta$, and $\alpha/\beta$ for theoretical cancer cells with $k = 1$ and $P_{0Q,2} = 0.2$.

*Competing interests*

The author declares that she has no competing interests.

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*Author's contributions*

Lydia Bilinsky did all work presented in this paper.

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