Therapies with Diverse Mechanisms of Action Kill Cells by a Similar Exponential Process in Advanced Cancers

Krastan B. Blagoev1,2, Julia Wilkerson3, Wilfred D. Stein3,4, James Yang5, Susan E. Bates3, and Tito Fojo3

Abstract

Successful cancer treatments are generally defined as those that decrease tumor quantity. In many cases, this decrease occurs exponentially, with deviations from a strict exponential being attributed to a growing fraction of drug-resistant cells. Deviations from an exponential decrease in tumor quantity can also be expected if drugs have a nonuniform spatial distribution inside the tumor, for example, because of interstitial pressure inside the tumor. Here, we examine theoretically different models of cell killing and analyze data from clinical trials based on these models. We show that the best description of clinical outcomes is by first-order kinetics with exponential decrease of tumor quantity. We analyzed the total tumor quantity in a diverse group of clinical trials with various cancers during the administration of different classes of anticancer agents and in all cases observed that the models that best fit the data describe the decrease of the sensitive tumor fraction exponentially. The exponential decrease suggests that all drug-sensitive cancer cells have a single rate-limiting step on the path to cell death. If there are intermediate steps in the path to cell death, they are not rate limiting in the observational time scale utilized in clinical trials—tumor restaging at 6- to 8-week intervals. On shorter time scales, there might be intermediate steps, but the rate-limiting step is the same. Our analysis, thus, points to a common pathway to cell death for cancer cells in patients.

See all articles in this Cancer Research section, "Physics in Cancer Research."

Cancer Res; 74(17); 4653–62. ©2014 AACR.

Introduction

In a landmark paper published in Cancer Research in 1964 that described a mouse leukemia model, Skipper formulated two laws of tumor kinetics that have been influential in designing treatment schedules and combination therapies (1, 2). The first law states that dividing cancer cells if left undisturbed grow exponentially. The second law states that when treated with a fixed amount of a therapy, the fraction of cancer cells killed remains the same irrespective of the total number of cancer cells. Although the cellular mechanism of the first law can be traced to symmetric cancer cell division, the molecular roots of the second law are still unknown. For example, as a tumor becomes smaller one could expect a larger fraction might be killed if a fixed amount of a therapy is being used, or if the drug is not uniformly distributed in a tumor, one may anticipate that some parts of the tumor will be more susceptible than others. The second law might also be in conflict with the fact that tumors are heterogeneous. Recent evidence highlighting the genetic diversity of tumors (3) suggests that the cells comprising a tumor have different susceptibilities to killing by a given therapy. Therefore, one might argue a less susceptible fraction is left behind, and the fraction of cells killed with the same dose should decrease with each consecutive drug administration. In agreement with Skipper (Fig. 1), we have previously shown through analysis of tumor measurements obtained from patients that tumor growth occurs exponentially and that the rate of this exponential growth is constant (4–8). The rates are far slower than those of the leukemia models used by Skipper, but the exponential nature of growth was confirmed. We have further shown that in patients with advanced metastatic cancer, the growth rate constant correlates well with overall survival. Although these data have additionally suggested that the regression of tumors is also exponential, we wished to examine other possibilities for the regression of tumor, given the existence of numerous proposed models of how therapies affect tumors. To do this, we mathematically modeled the occurrence of cancer cell death in several ways and analyzed tumor measurements or biomarker data from clinical trials in which a diverse group of anticancer agents was studied in advanced cancers. In analyzing data from these trials, we will show that although the therapies are mechanistically different, ranging, for example, from microtubule-targeting agents to activated T cells, in all
Figure 1. From ref. 1, “Chart 4”—hypothetical illustration of the possible importance of drug level and schedule in attempts to achieve “cell cure” in early experimental leukemia (L1210) animals. A, untreated controls; number of leukemic cells quadruples daily until the number reaches about $10^9$, at which time the animals die. B, daily drug treatment; low level, long term (until death); plotted to represent a daily 50% “drug kill” of the animals’ leukemic cell population and a daily quadrupling of the surviving leukemic cells. Increase in host life span was achieved, but “cell cure” was not approached. C, daily drug treatment; moderate level, long term; plotted to represent a daily 75% “drug kill” of the animals’ leukemic cell population; in finite host survival if cumulative drug toxicity, development of drug resistance within the leukemic cell population or the blood–brain barrier problem did not intervene. D, daily drug treatment; high level, short term; plotted to represent a daily 99% “drug kill” of the animals’ leukemic cell population, no other complications, and “cure” of a $10^5$ cell inoculum. In our actual experience, a single maximum dose has been most effective with this very rapidly proliferating experimental disease. Adapted from Skipper (1).
cases, the sensitive tumor cells disappear exponentially. We propose, based on general mathematical grounds, that there is a common feature by which cells die when treated with anticancer agents.

Results

We present three theoretical models: (i) a "constant fraction or exponential model," where a constant fraction of cells is killed with each drug administration; (ii) a "constant number model," where a constant number of cells are killed with each treatment; and (iii) a "tumor surface model," where only (principally) cells on the surface of the tumor are killed with each treatment (Fig. 2). All three models can be analyzed using a simple mathematical approach.

To obtain a relation between the number of sensitive cells and time, we can express at some point of time the relation between the number of sensitive cells at that time and at a time shortly before that time. If the number of cancer cells in the tumor is \( N \) then

\[
N(\text{at time } t + \Delta t) = N(\text{at time } t) - N_{\text{killed}}(\text{between time } t \text{ and } t + \Delta t),
\]

where \( N_{\text{killed}} \) is the number of cells killed by a therapy during time interval \( \Delta t \). For the three models then, we have the following:

- Constant fraction or exponential model: \( N_{\text{killed}} \) in any time interval \( \Delta t \) during treatment is \( \Delta t \cdot f \cdot N \), where \( f \) is a fraction of the total number of cells (\( N \)) in the tumor at time \( t \). The fraction \( f \cdot N \) is the number of cells killed per unit time and is proportional to the total number of cells in the tumor.
- Constant number model: \( N_{\text{killed}} \) in any time interval \( \Delta t \) during treatment is \( \Delta t \cdot C \), where \( C \) is the number of cells killed per unit time. \( N_{\text{killed}} \) in time interval \( \Delta t \) is constant and is "independent of the number of cells in the tumor," being dependent only on the time interval \( \Delta t \).
- Tumor surface model: \( N_{\text{killed}} \) is the number of cells on the surface of the tumor and if we assume that the tumor is spherical we can obtain an expression for the relation between the fraction of cells on the surface of the tumor and the total number of cells in the tumor (see the Supplementary Information).

One can cast the three models in a differential form by using \( \Delta N \) to mean the change in cell number during time \( \Delta t \), with \( N \) as the total number of cells at the outset. The equations describing the three models are:

- Constant fraction or exponential model, where the same fraction of cells is killed with each treatment and the number is that fraction:
In the constant fraction or exponential model, the (sum of the) longest diameter of the tumor decreases as the cubic root of time.

In the tumor surface model, the (sum of the) longest diameter of the tumor decreases linearly in time.

Given the above theoretical models, we examined data from a diverse group of clinical trials to investigate how different therapies affect tumors—specifically how these different therapies bring about regression of tumors. In most patients, the tumor first shrinks at the start of treatment and after reaching a minimum, increases in size, as drug resistant cells comprise an increasingly larger fraction of the tumor. We have shown and reported previously that growth of the resistant fraction is well modeled as an exponential, which we have estimated with the growth rate constant, \( g \) (4–8). Whether the resistant fraction preexists before therapy begins, or resistance is acquired after therapy has begun, there will be a time when the sensitive cells are continuing to be killed while the resistant cells are growing. The exponentially growing fraction of cells also exists during that part of the time course, corresponding to a decrease in the overall tumor size—a decrease that is occurring because the quantity of tumor being killed is still greater than the quantity growing exponentially, so the net result is the reduction in size. Because these two processes are occurring concurrently, we include in our analysis of the killing process, the exponentially growing fraction in each model as described below. The equations that describe the tumor volume (number of cells) and the longest diameter of the tumor are shown in Table 1.

The constants \( \phi, d, \) and \( g \) are estimated from the data of quantities of tumor obtained while a patient receives a given therapy. The data are either measurements of the longest diameter of tumor masses seen radiographically or the values of serum markers recorded as treatment is administered and response is assessed. Note that the exponential model is described by the same equation whether volume or diameter is used. The fraction or proportion of the initial tumor that is sensitive to treatment and will eventually decay or regress is represented by \( \phi \) and is to be distinguished from the fraction that is killed with each cycle of treatment. The decay or regression rate constant represented by \( d \) describes the rate at which the fraction of tumor that is sensitive to the therapy is disappearing. The growth rate constant represented by \( g \) describes the rate at which the fraction of tumor that is resistant or relatively resistant to the therapy is growing, and as we have discussed, this growth occurs exponentially. Finally, the fraction of the initial tumor that is resistant or relatively resistant to the therapy is equal to \((1 - \phi)\), given that \( \phi \) has been defined as the fraction of the initial tumor that is sensitive to the therapy. All the analyses of the killing process include the exponentially growing fraction \((1 - \phi) \cdot e^{dt}\), where \((1 - \phi)\) is the fraction of the tumor resistant or relatively resistant to the therapy in each model. Although the constant fraction or exponential model represents the exponential decrease we have reported before (4–8), the other two models—constant number and tumor surface—represent nonexponential models.

\[
\frac{\Delta N}{\Delta t} = -\text{number} \cdot N
\]

\[
\frac{\Delta N}{\Delta t} = -\text{number}
\]

\[
\frac{\Delta N}{\Delta t} = -\text{number} \cdot N^{2/3}.
\]

We have assumed that the rate at which a therapy kills cells does not vary with time. This might not be the case. For example, combination therapies with antiangiogenic drugs that possibly normalize the vasculature should be expected to lead to changes in the rate of killing. Reducing angiogenesis could starve the tumor of nutrients and also have a kill effect on the delivery of a therapy, thus slowing the kill rate, or normalization of leaky blood vessels with a decrease in their tortuosity could improve blood flow so that delivery of a therapy to the tumor improves, increasing kill rate.

The number of cells in a tumor correlates with its volume. Given this, the solutions of the equations describing the three models are displayed in Fig. 3A:

- In the constant fraction or exponential model, the volume decreases exponentially with time.
- In the constant number model, the volume decreases linearly with time.
- In the tumor surface model, the volume decreases as the third power.

Often the sum of the longest measured linear distances or the longest diameters of the radiographically assessed tumor are used as a criterion for response as described in RECIST. In this case, the solutions of the equations describing the three models are displayed in Fig. 3B:

- In the constant fraction or exponential model, the (sum of the) longest diameter still decreases exponentially but with one third of the rate.
The analysis of clinical data is summarized in Table 2 (9–17). The measurements in the case of prostate cancer and multiple myeloma are "volumetric," because serum PSA (in patients with prostate cancer) and M protein levels (in patients with multiple myeloma) were used as the measures of tumor quantity. The rest of the trials used RECIST guidelines to assess efficacy, so that the sums of the longest linear diameters of the tumor masses assessed radiographically were the

Figure 3. A, graphs depicting the solutions of formulas (for constant fraction or exponential model, constant number model, and tumor surface model), where tumor volume (or number of cells) is assessed. B, graphs depicting the solutions of the same formulas as in A, where the longest linear distance or longest diameter of the tumor is assessed.
Table 1. Equations describing the tumor volume and the longest diameter of the tumor

<table>
<thead>
<tr>
<th>Model</th>
<th>Exponential regression model</th>
<th>Nonexponential (Ne) regression models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells killed</td>
<td>Constant fraction</td>
<td>Constant number</td>
</tr>
<tr>
<td>Volume</td>
<td>( \phi \cdot e^{-dt} + (1 - \phi) \cdot p^{gt} )</td>
<td>( \phi \cdot (-d \cdot f) + (1 - \phi) \cdot p^{gt} )</td>
</tr>
<tr>
<td>Diameter</td>
<td>( \phi \cdot e^{-dt} + (1 - \phi) \cdot p^{gt} )</td>
<td>( \phi \cdot (-d \cdot t) + (1 - \phi) \cdot p^{gt} )</td>
</tr>
</tbody>
</table>

Discussion

We have discussed several models that describe how cell death could occur when a treatment is applied to a cancer. Analysis of data from various clinical trials suggests that models (equations), in which the regression or decay of tumor occurs exponentially, best describe the rate of cell death in human tumors. These results establish, in a diverse group of human cancers treated with a diverse group of therapies, the validity of Skipper’s original observation in an L1210 leukemia model, albeit at a much slower rate (1, 2). An exponential decrease in the quantity of tumor cells means that with each successive treatment the same fraction of cells is killed every time.

Exponential decay is observed in one step in radioactive decay, where the probability for decay of an unstable element to another stable element is unchanged as time passes (18). If the decay is not exponential, either the substance is a mixture of elements that have different decay rates or the elements decay into intermediate states, which in turn decay to the final stable element. In this latter case, the decay is not exponential in time, but a sum of exponentials on a time scale. If all transition rates are the same, the model can be solved explicitly as shown in the Supplementary Information. In this case, the solution is described by a power of time multiplied by an exponential (19). Another biologic phenomena in which a fractional or exponential kill occurs is with antibiotics and bacteria (20). In this case, the quantity of viable bacteria also decreases exponentially with each drug application. In contrast, nonexponential laws, reflecting the existence of more than one rate-limiting process have been observed in the case of the age dependence of cancer incidence (19, 21). In this case, more than one gene mutation is needed for cancer to occur and the incidence curves are not exponential but rather are described by power laws.

The data analyzed here, showing tumor regression in most cases occurs exponentially, argues against the existence of an intermediate step in the process of cell death. This does not mean cells that are damaged cannot repair their damage and rejoin the viable fraction. This is shown in Fig. 5, where each treatment damages some cells, a fraction of which die while the remaining repair the damage and rejoin the viable fraction. In the latter case, if the fraction that repairs the damage is constant, then cell death occurs exponentially. It also does not mean only one molecular event occurs before cell death. A complex set of events might take place, but these must always lead to cells that are either fully viable or dead, that is, the ensuing treatment begins from the same initial state as the previous treatment administration. That there is no intermediate step may be a consequence of the doses used with cancer therapies; if these doses are always administered at high enough amounts, then the different steps required for cell death are accomplished within one treatment. This seems unlikely given the observation of exponential cell death with a myriad of drugs and with modalities other than drugs. But if the reason an intermediate step is not "detected" is because doses are excessive, then a deviation from exponential or fractional cell death would be an indication the treatment is insufficient in quantity.
Table 2. Tabulation of which equation best fit individual clinical datasets

<table>
<thead>
<tr>
<th>Cancer histology</th>
<th>Therapy</th>
<th>N</th>
<th>dx</th>
<th>gx</th>
<th>gd</th>
<th>g + d + f</th>
<th>Of total</th>
<th>Of regression</th>
<th>Data for which best-fit model was an exponential regression (or growth) model</th>
<th>Data for which best-fit model was a nonexponential regression model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>Thalidomide + Taxotere (9)</td>
<td>55</td>
<td>5</td>
<td>12</td>
<td>17</td>
<td>15</td>
<td>89.1%</td>
<td>86%</td>
<td>Exponential model</td>
<td>Nonexponential models</td>
</tr>
<tr>
<td>RCC</td>
<td>Bevacizumab (10)</td>
<td>34</td>
<td>1</td>
<td>3</td>
<td>18</td>
<td>9</td>
<td>91.2%</td>
<td>90.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>ATTP/ARTP (11)</td>
<td>98</td>
<td>8</td>
<td>13</td>
<td>27</td>
<td>44</td>
<td>93.9%</td>
<td>92.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>Capecitabine + Irinotecan (12)</td>
<td>211</td>
<td>40</td>
<td>5</td>
<td>100</td>
<td>37</td>
<td>86.3%</td>
<td>85.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>Capecitabine (12)</td>
<td>160</td>
<td>33</td>
<td>17</td>
<td>64</td>
<td>16</td>
<td>81.3%</td>
<td>79%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>Bortezolom ± Dexamethasone (13)</td>
<td>259</td>
<td>57</td>
<td>17</td>
<td>75</td>
<td>81</td>
<td>88.8%</td>
<td>88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCC</td>
<td>Ketoconazole + Amlodipine (9)</td>
<td>67</td>
<td>5</td>
<td>17</td>
<td>23</td>
<td>18</td>
<td>94%</td>
<td>92%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCC</td>
<td>Interferon (15, 16)</td>
<td>210</td>
<td>48</td>
<td>37</td>
<td>71</td>
<td>20</td>
<td>83.8%</td>
<td>79.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCC</td>
<td>Sunitinib (15, 16)</td>
<td>300</td>
<td>56</td>
<td>8</td>
<td>153</td>
<td>60</td>
<td>92.3%</td>
<td>92.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>Vaccine</td>
<td>67</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>82.1%</td>
<td>45.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCC</td>
<td>T-cell-based immunotherapy</td>
<td>62</td>
<td>8</td>
<td>6</td>
<td>26</td>
<td>11</td>
<td>82.3%</td>
<td>80.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCC</td>
<td>T-cell-based immunotherapy</td>
<td>51</td>
<td>2</td>
<td>2</td>
<td>23</td>
<td>10</td>
<td>82.4%</td>
<td>81.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTC</td>
<td>Vandetanib (17)</td>
<td>118</td>
<td>45</td>
<td>7</td>
<td>98</td>
<td>16</td>
<td>88.3%</td>
<td>87.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>Various</td>
<td>2,027</td>
<td>361</td>
<td>203</td>
<td>795</td>
<td>427</td>
<td>88.1%</td>
<td>86.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Each patient dataset appears only once under the best-fit model (formula) for that particular data. Datasets with four or more points were used in the analysis. The eight models were fit for each dataset. P-value for the fit had to be <0.1. To select the best model for those with more than one valid fit, the AIC was calculated for each model. AIC = 2k - 2lnL, where k is the number of parameters and lnL is the log likelihood; the preferred model was selected as the one that minimizes the AIC.

Cancer histology, assessment methods: aSerum PSA; bRadiographic imaging, CT or MRI; cM-spike, immunoglobulin levels.

Formulas: See text for details. [dx, data show only regression and no evidence of growth; gx, data show only growth and no evidence of regression].

\[dx; f(t) = \exp(-d \cdot t) - 1; gx; f(t) = \exp(g \cdot t) - 1; gd; f(t) = \exp(-d \cdot t) + \exp(g \cdot t) - 1; g + d; f(t) = \delta \cdot (1 - \exp(-d \cdot t)) \cdot \exp(g \cdot t)\]

\[Ne1 = (\delta - d \cdot t) + (1 - \delta) \cdot \exp(t^2); Ne2 = (\delta - d \cdot t) + (1 - \delta) \cdot \exp(t^2); Ne3 = (\delta - d \cdot t) + (1 - \delta) \cdot \exp(t^2); Ne4 = (\delta - d \cdot t) + (1 - \delta) \cdot \exp(t^2)\]

The percentage of calculations: \(N = (dx \cdot gx) + (gd \cdot (g + d + f)) + (Ne1) + (Ne2) + (Ne3) + (Ne4) + \text{none}\).

The percentage of total (exponential regression models) = (dx) + (g + d + f) + (gd) + (dx + gd + f).

The percentage of decay (exponential regression models) = (dx) + (g + d + f) + (gd)(N - (gd)).

The percentage of total (nonexponential regression models) = (Ne1) + (Ne2) + (Ne3) + (Ne4)(N - (gd)).

The percentage of decay (nonexponential regression models) = (Ne1) + (Ne2) + (Ne3) + (Ne4)(N - (gd)).

Abbreviations: Ne, nonexponential; none, data did not fit any model; \(\delta\), fraction of tumor that is sensitive to the therapy being administered; RCC, renal cell carcinoma; MM, multiple myeloma; MTC, medullary thyroid carcinoma; ATTP, Avastin (bevacizumab); Thalidomide + Taxotere + Prednisone; ATTP, Avastin (bevacizumab) + Revlimid (lenalidomide) + Taxotere + Prednisone; Doxil, liposomal doxorubicin.
Our data also imply that although a tumor may be composed of cells harboring different mutations and expressing crucial genes at different levels, the cells in tumor die through a common pathway. This conclusion is reached, because if different pathways existed and the rates of cell death for each of pathways were different, the decrease of the tumor quantity would be nonexponential. This does not mean cells in all tumors must die in the same way. Cells in a lymphoma or a testicular cancer may die as a result of apoptosis, whereas cells in another solid tumor die via a different pathway. Alternately all cancer cells in all tumors could die via apoptosis, only at varying rates. In this scenario, the rate-limiting step could be cytochrome C release or caspase activation. Thus, the original observations of Skipper on the L1210 leukemia model suggesting meaningful increases in survival depended on increasing the fraction of tumor cells that die following treatment is more relevant for solid tumors than we have considered in recent years. If we...
kill the same fraction of tumor cells at each step, the data imply that we must increase that fraction—a very old concept, but one now supported by actual patient data.

In summary, we have discussed several models of cell death following treatment of human cancers. We have analyzed data from a diverse group of clinical trials and demonstrate that in patients treated with diverse therapies, cell death occurs preferentially in an exponential manner. A corollary of this exponential cell death is that there can be no intermediate with a significantly different rate-limiting step in the occurrence of cell death; cells are not incrementally damaged by successive therapies but rather, having been damaged by a therapy, either die or repair the damage. A second corollary is that cell death in a tumor follows a common pathway. Together with previous analyses demonstrating exponential growth as the preferred pattern of tumor growth, we hope this information can be used to better understand how therapies work and how to make them better.
Disclosure of Potential Conflicts of Interest
S.J. Bates received a commercial research grant from Celgene. No potential conflicts of interest were disclosed by the other authors.

Disclaimer
The National Science Foundation had no role in study design, data collection, and analysis, decision to publish, or preparation of the article. The views presented here are not those of the National Science Foundation and represent solely the views of the authors.

Authors' Contributions
Conception and design: K.B. Blagoev, A.T. Fojo
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.J. Yang, A.T. Fojo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.B. Blagoev, J. Wilkerson, S.J. Bates, A.T. Fojo

References