Order-of-magnitude Estimates of Latency (Time to Appearance) and Refill Time of a Cancer from a Single Cancer 'Stem' Cell Compared by an Exponential and a Logistic Equation

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Abstract. *Background/Aim: The time required before a mass of cancer cells considered to have originated from a single malignantly transformed cancer 'stem' cell reaches a certain number has not been studied. Applications might include determination of the time the cell mass reaches a size that can be detected by X-rays or physical examination or modeling growth rates in vitro in order to compare with other models or established data. Materials and Methods: We employed a simple logarithmic equation and a common logistic equation incorporating 'feedback' for unknown variables of cell birth, growth, division, and death that can be used to model cell proliferation. It can be used in association with free or commercial statistical software. Results: Results with these two equations, varying the proliferation rate, nominally reduced by generational cell loss, are presented in two tables. The resulting equation, instructions, examples, and necessary mathematical software are available in the online appendix, where several parameters of interest can be modified by the reader www.uic.edu/nursing/publicationsupplements/tobillion_Anderson_Rubenstein_Guinan_Patel1.pdf. Conclusion: Reducing the proliferation rate by whatever alterations employed, markedly increases the time to reach 10^9 cells originating from an initial progenitor. In thinking about multistep oncogenesis, it is useful to consider the profound effect that variations in the effective proliferation rate may have during cancer development. This can be approached with the proposed equation, which is easy to use and available to further peer fine-tuning to be used in future modeling of cell growth.*

We present a brief review of cell growth kinetics as a background for the assumptions of the equations offered in the online appendix [www.uic.edu/nursing/publicationsupplements/tobillion_Anderson_Rubenstein_Guinan_Patel1.pdf]. Normal or malignantly transformed stem cells are believed to undergo two forms of cell division: symmetric or asymmetric (1) (Appendix, Table I A-C). Symmetric cell division generates two 'identical' cells, either stem or daughter cells; asymmetric division generates one stem and one daughter cell. The relation between these two patterns of proliferation and the extent to which they can be 'blended' seem obscure. Factors that determine which stem cells continue to replicate or are withdrawn into a 'reserve' have not been identified, but an important role is assigned to the niche in which they reside (2). Retention by 'parental' stem cells of a 'conserved' DNA and transfer of newly synthesized DNA strands to daughter cells subject the former to fewer mutagenic events from errors inherent in DNA replication (3). Daughter cells entering the transit-amplifying cell population proliferate for a time while undergoing various degrees of partial differentiation. Estimates of the number of stem cells in different experimental and clinical types of cancer have ranged from <1% to >30%, depending upon the methods and criteria used to identify them (4).

Having served as the original source of proliferating malignant daughter cells, replicating cancer stem cells (CSCs) augment cancer growth by increasing the pool of transit-amplifying daughter (D) cells. Gompertzian kinetics (5), in which the exponential growth rate of enlarging tumors eventually decreases, partly depends on a reduced rate of proliferation and increased cell death.

According to the stochastic CSC model, potentially any cell may develop into a CSC (6), while in the stem cell model, CSCs originate from resident non-transformed tissue

stem cells (7). For our purposes, the terms 'cancer' and 'tumor' have been used interchangeably, although the latter can be benign or malignant.

Development of Many if not Most Solid Carcinomas is not Linear but is Interrupted by Prolonged Periods of Relative Quiescence

The development of many solid types of cancer, including those of the breast, colon, prostate, and pancreas, occurs over a number of years (8-10). A core of driver genetic and possibly epigenetic events accompanied by numerous progressor (10) or 'passenger' mutations that may not directly contribute a growth advantage are present before the final development of a CSC unresponsive to the constraints on normal stem cell proliferation or limited by the requirements of their tissue or organ of origin. Where their development is largely stochastic, built-in delays in oncogenesis seem explicable. The stem cells considered in the assumptions for our equations are near or at the end of their development and exhibit inappropriate self-renewal.

The periods of delay followed by accelerated growth periods are diagnostically 'silent,' presently excluding detection of solid breast, colon, lung, and prostate cancer during a time when they could be curable. The development of pancreatic cancer involves an estimated decade between the initiating mutation(s) and development of the non-metastatic 'founder' cell (10, 11). The original founder mutation might depend upon stochastic or hierarchically determined events. Some five more years are required for a metastatic capacity to develop. A series of mutations are associated with these events, occurring during the intervals of slow development. These genetic and epigenetic changes may also be associated with a gradual transition from an epithelial to a more mesenchymal-like phenotype as the cell population develops from less benign to a malignant stem cell (12).

Assumptions Regarding Estimates of Cancer Cell Growth

Estimates of the time required to generate a population from one initial malignantly transformed cell or for 'refilling' a treated tumor depend upon the assumptions chosen to describe the responses of cell division, cell death, and their relation to the transit-amplifying population. The extent to which CSCs and daughter cells continue to proliferate is not known. An average cell cycle time of about 24 hours is assumed, based on data from cultured cells. Cell cycle times from 40 to 100 hours have been estimated in transplanted human tumors and in human carcinomas.

The simplest exponential power relation, $N=2^r$, which on logarithmic transformation is $\log(N)=r\log(2)$, has been used to estimate the number of generations (r) required to generate N number of cells originating from an initial cell, in this case imagined to be a CSC. Most of the stem and daughter cancer cells envisioned in this study are considered near or at the end of their malignant development and capable of maximum rates of proliferation. During a protracted development of the oncogenic process, precursor cancer cells with reduced proliferation and depletion rates are likely, and these may exhibit extended periods of slow growth for a number of reasons.

Logistic equations represent a more sophisticated approach to describing the geometric growth of populations. A typical logistic equation includes terms representing 'feedback' that alters initial and terminal rates of proliferation. The general rate of growth of any population (a microbial colony, for example) can be described as: $dN/dt= KxN – rxN^2$ for both K and $r=1>0$ (13,14). KxN is the net growth rate: the excess of births over deaths. The rxN^2 term introduces a non-linearity, but the same limitations remain due to the lack of quantitative information about unavailable parameters.

Order-of-Magnitude Estimates of the Time to Appearance and Tumor Cell Refill Times by the Exponential (Geometric) Growth Function

It is generally accepted that 1 mm³ of tissue originating from a single cell after 20 doublings contains about 10^6 cells, after 27 doublings, some 0.5×10^9 cells are contained in a 0.5 cm³ mass, and after 30 to 32 doublings, about 10^9 cells can be found (9).

The logarithmic relationship $\log(N)=r\log(2)$, estimates the number of generations to yield N cells from a single CSC, specifically 10^9 cells occupying 1 cm³, a volume that can be detected by careful physical examination. N could be considered to approximate 2^r, where $r=p–d$, where p is the proliferation rate and d represents reduction of p due to cell morbidity. An accelerated rate of proliferation of transit-amplifying cells (which cannot be accounted) would increase the apparent overall proliferation rate and reduce the time to accumulate 10^9 cells. Initially, all or most daughter cells remain in cycle but their activity diminishes as they partially differentiate or die. A probably much less common dedifferentiation from daughter cells to CSCs has been suggested (15). In C57BL/6 mice chronically infected with *Helicobacter*, repopulation of the stomach with bone marrow cells which progress through metaplasia to intraepithelial cancer has been reported (16).

Table IA shows exponential model estimates of generation and refill times with proliferation rates of $r=1, 0, 5, 0.2, 0.1$ and 0.01 (equivalent to 100, 50, 20, 10, and 1%). An 'effective' proliferation rate can be achieved in various ways. If 55% of daughter cells and CSCs (expressed as a percentage of $p=1$) are considered to have cycled in each

Table I. *Estimates of generation and 'refill' times according to a simple exponential model. One cell cycle generation time is taken as 24 hours. These data were in agreement, whether generated by the simple computer program in the Appendix or with a Texas Instruments T1 30×. As mentioned in the text, reasons for p (proliferation rate) variation may be related to a greater or lesser extent (or not at all) to r, or to a variety of other factors; some suspected, most unknown.*

A: Days to generate 10 cells.

$r=(p-d)$	Days
1.0	29.9
0.50	59.8
0.20	149.5
0.10	299.0
0.01	2990.0
	(8.2 years)

B: Days to 'refill' to 10^9 cells from generation #3 (8 cells).

$r=(p-d)$	Days
1.0	26.9
0.50	53.8
0.20	134.5
0.10	269.0
0.01	2659.0
	(7.3 years)

C: Days to generate 0.5×10^9 cells.

r	Days
1.0	28.9
0.5	57.8
0.2	144.5
0.1	289.0
0.01	2890.0
	(7.6 years)

r=number of generations. *p*=proliferation rate. *d*=reduction of *p* due to cell morbidity. Symmetric and asymmetric proliferation values are considered identical, as we are unable to distinguish between them.

Table II. *Nominal time to generate or to 'refill' N number of cells, according to a representative logistic model. The maximum proliferation rate is obtained from the equation or the graph. The maximum growth rate and the time to 10^9 cells are obtained from the solved equation or the cell print-out available in the online Appendix.*

A: Days to generate 10^9 cells.

$r=(p-d)$	Days
1.0	38
0.5	76
0 2	188
0.1	376
0.01	3736 (10.2 years)

B: Days to refill to 10^9 cells from 8 cells (generation #3).

$r=(p-d)$	Days
1.0	36
0.5	72
0 2	180
0.1	360
0.01	3600
	(9.7 years)

C: Days to generate 0.5×10^9 cells[a].

r=(p–d)	Max. prolif rate.	Days to 0.5×10^9 cells
1.0	19	43.5
0.5	40	91.0
0.2	100	227.0
0.1	201	452.0
0.01	2004	4500.0
		(11.7 years)

r=number of generations. *p*=proliferation rate. *d*=reduction of p due to cell morbidity. [a]Notice the difference in proliferation rate *t* due to a greater *r*.

generation and 5% of all proliferating cells die while the remainder are differentiated and non-proliferative, the estimated number of generations with a 24-hour (one generation) cell cycle time to reach 10^9 cells from one initial CSC would approximate $2^{0.50r}$. Taking the natural logarithms of both sides yields an answer of 59.8 days. Manual solution of these simple equations is as follows. For convenience, common (Briggsian) logarithms to base 10 rather than natural (Naperian) logarithms to base e=2.71828 were used. $N=2^r$; $10^9=2^r$; log $10^9=r\log_{10}2$; 9=0.31r; r=29.9 days. For $10^9=2^{0.5r}$, 9+0.5×0.391=59.8. $0.5\times10^9=2^r$, $\log_{10}109=r\times\log_{10}2+\log_{10}2$, 9-$\log_{10}2$=0 301r or r=28.9 days. Asymmetric or symmetric proliferative patterns generate similar results because of the nature of the logarithmic relationship, the

assumptions chosen, and the lack of information regarding the altering behavior of CSCs and daughter cells over time. (Note that the other outcomes in the table vary proportionally to whatever *r* value has been used to calculate the result.) As the original assignment of CSCs and daughter cells changes over time, their ratios under varying symmetry or asymmetry would differ, depending upon circumstances (Appendix, Figure 1A-C). The differing blended patterns result in a potential form of feedback as the ratio of CSC to daughter cells changes with increasing cell numbers (Appendix, Figure 1B,C).

Having established the time to generate 10^9 cells from a single CSC under various conditions of *r* (Table IA), how many fewer days would be required following an effective therapy for only 8 CSCs (three days' worth of growth) to repopulate the tumor to 10^9 cells? Whether subtracting 3 days from the

values in Table IA or calculating the rate of proliferation starting with 8 cells, the results are the same (Table IB). Finally, noting that 0.5×10^9 cells, a quantity detectable by x-rays, are only one day ($r=1$) away from generating 10^9 cells ($p=1$, $d=0$), subtracting one day from the corresponding value in Table IA generates the corresponding component of Table IC. For other values of r, dividing '1 day' by the factor representing r and multiplying the number of days when $r=1$, as with $r=0.5$, subtracting 2 days from the values in Table IA generates that value, 53.8 days, for Table IC. Alternately, values can be directly calculated from the basic formula, with 0.5×10^9 cells as the end point. Once again, the two approaches give corresponding values. As noted, changes in r, the 'effective' proliferation rate, profoundly extend the time before detectable numbers of cancer cells develop.

Time to Appearance and Time to Refill Estimated with a Logistic Equation

With the logistic equation, the situation is very different. The maximum rate of proliferation and the time to appearance are obtained either graphically or from the printed tables available in the Appendix (the latter in an abbreviated version is presented in Table II). Note again that once any single value is available, the others can be approximated as described above. Sections A and B of exponential Table I and logistic Table II are both internally and mutually consistent. The exponential equation generates more cells per unit of time than does the logistic equation. However, both equations gave unanticipated results in which the values in section C exceeded those of section B (Table I) or both A and B (Table II).

Reasons for these quantitative differences relate to the steady exponential rise compared to the 'interrupted' logistic growth rate with the gradual increase to a maximum that is followed by a more rapid decline of the latter (see Appendix). While it may not be intuitively apparent that beginning with 8 cells, 10^9 cells are reached in a little less time than an initial single cell reaches 0.5×10^9 cells, such is the nature of exponential growth under these assumptions. The more 'mixed' unfolding of a logistic equation, initially a very delayed exponential growth, but which is subsequently attenuated, yields an initially unanticipated result.

Elements of a Logistic Model for a Stem Cell Application

Logistic equations are commonly used to model laboratory or clinical cancer growth or their hypothetical simulations (18-22). To provide a more realistic model of tumor growth, logistic equations incorporating a reduction in proliferative rate toward the end of cancer cell accumulation by including a term representing feedback that dampens later growth rates have been widely employed (5, 17).

As before, stem cells can be considered in an exponential model including two types of processes: the proliferation and depletion rates for CSCs and daughter cells. This simplified biological model can be further developed to approximate the intrinsic behavior underlying the biological behavior. The final equation is:

$$N(t)=K \times N(0)/\{N(0) + (K–N(0) \times \exp(−rxt)\}$$

as discussed further in the online Appendix. (Note: K, the carrying capacity, is denoted as N in the exponential model.) The results of duplicating the analyses using the R program (The R 2.13.1 program for Windows (32/64-bit) is available at htpp://cran.r-project.org/bin/windows/base/) with the results generated from the simpler exponential equation starting under the same initial conditions [one initial CSC, $r=(p–d)$ of 1.0 and various cell depletions (or alternately holding $d=0$ and varying p alone)] are presented in Table II. As anticipated, the times to reach the notational cell numbers obtained from the logistic equation, Sections A and B, were greater than those of the exponential equation. This was true at all values of K chosen for the 'time to appearance,' 'time to refill' and 'time to generate 0.5×10^9 cells.' The accepted number of localized cancer cells considered detectable by conventional x-rays can be generated in a number of ways within the boundaries of $1 > 0$. Values of $r=0-1$ can be considered to represent reduction in cell numbers, while $r > 1$ can be considered to reflect enhanced proliferation of daughter cells due, for example, to a reduced cell cycle time (a number that is unavailable). Since logistic equations rise to a maximum rate and subsequently essentially reverse themselves due to the feedback term, the times to accumulate 10^9 or half that number of cells are available in the numerical printout from the logistic program available in the online Appendix [www.uic.edu/nursing/publicationsupplements/tobillion_Anderson_Rubenstein_Guinan_Patel1.pdf] where instructions regarding its use are also provided. Graphical estimation of these numbers is more problematic, due to the prolonged periods with little apparent change present at the beginning and end of the accumulation (see the Figure in the Appendix).

With the logistic equation, the time (in days), when the maximum growth rate and carrying capacity N is reached, is obtained from the numerical and graphic output for each, as generated in the SPSS or R statistical programs.

Use of Programs in the Appendix to Duplicate and Extend these Determinations

The population growth of tumors exhibits a saturation limit. This property is well reflected in the S–shaped sigmoid curve. The controlling biological parameters are proliferation rate, depletion rate, and saturation. In our case, 10^9 cells is termed the carrying capacity. To portray a visual image and a numerical solution for this biological process, commands

are copied and pasted into *R* or SPSS. The program TOBILLION.R has the necessary controls for interested users to provide their own choice of values for the three variables.

There are two important considerations in running a logistic model: (i) the size of the carrying capacity (*K*), as large values can require experimentation before graphic and numerical solutions are obtained, and (ii) how to know at what point the system reaches maximum growth, before the rate starts its decline. Selection of appropriate 'beggen' and 'endgen' values is required to allow correct visualization of the graphical and enumeration of the numeric output. It is also important to note that these equations work with natural logarithms e or base 10, whichever the case may be.

As a simple hypothetical example, say one is interested in starting from a single cell No as 1 with a carrying capacity of reaching 100 cells (*i.e.*, K=100). With the proliferation rate as 1 and depletion rate of zero, prolifer=1, deplet=0. Furthermore, one can study the loop up to the 20th Generation. With these changes in the program, one can copy from ###BEGIN to ###END and paste into *R*, which gives the digital and graphical output. The necessary programs are included in the Appendix, and *R* can be downloaded from the Internet. The graph or table indicates that maximum growth is reached at the 5th Generation.

The program was focused on the carrying capacity to reach either 10^9 cells or half that number, which can be detected respectively by physical or x-rays examination. Altering the input parameters allows other combinations to be generated.

Discussion

We were interested in comparing a simple exponential equation with a more complex logistic equation, to obtain order-of-magnitude estimates of time required for the growth from an initial CSC to quantities of tumor sufficient for detection by physical or x-rays examination. These are available in the online appendix [www.uic.edu/nursing/publicationsupplements/tobillion_Anderson_Rubenstein_Guinan_Patel1.pdf]. Comparing different rates of cell proliferation and loss might provide a useful sense of the tempo of cancer cell accumulation. We expected that the times calculated from the exponential equation would be considerably shorter than those from the logistic equation (with its initially slower and diminishing terminal rate of growth), as proved to be the case.

Despite the inability to include many of the components required for a more complete simulation, these two simple programs provide a sense of order-of-magnitude changes in the tempo of cancer growth that can be exhibited by solid tumors subjected to different assumptions of proliferation and cell loss. Interestingly, delay between an ability to reach

0.5×10^9 cells (detectable by x-rays) and 10^9 cells (palpable by physical examination) could be short, a property of exponential growth.

Estimates of the time to appearance with *r*=1, *p*=1 and *d*=0 as the index example were considered to involve fully developed malignant cancer cells in optimal niches. Since many types of solid cancer develop over a number of years (8-10), 'premalignant' precursors lacking the necessary genetic or epigenetic changes could not be considered. Cancer development is believed to occur from partially transformed daughter cells sequentially acquiring additional 'driver' genetic or epigenetic events (11), necessary for the further development of malignant properties. The relation between proliferation rates and the probability of developing the subsequent required oncogenetic changes, both probably contribute to delay in the development of the next necessary genetic driver event (3, 11, 23, 24). Whether CSCs originate through a stochastic process or by a more linear development from an initial stem cell was not part of the assumptions.

For the very approximate estimates of these parameters and under the conditions chosen, the simple exponential equation with *r* equal to 1 initially would reflect the behavior of a very rapidly growing malignancy; the more complicated logistic equation characteristic would reflect a slower growing one subject to limitations on its proliferation. Exponential equations may more closely mimic some rapidly growing hematopoietic malignancies, while logistic equations characterize non-hematopoietic cancer. Differences between these malignancies in their interaction with the vascular system may contribute to their growth rates. A discrete role for CSCs as distinct from their daughter cells could not be considered, as suppositions about their behavior over time were not considered (25).

With logistic equations, as the carrying capacity *K* (end number of cells sought) becomes large and the growth rate *r* diminishes to very low values, replication of the initial and final cell(s) is represented as extending over many cell generations before it is completed. This amounts to an extremely protracted cell cycle over a number of generations (days) that delays completion of the initial and final cell divisions.

Logistic equations subject to certain constraints can be reduced to exponential equations. This may occur if *K* becomes very large and the $N[t]/K$ term becomes sufficiently reduced or if the population size $N[t]$ were much smaller than the carrying capacity *K*. Under these conditions, the population can grow nearly exponentially until the population size is no longer much smaller than *K*.

The history of an evolving tumor resembles a variation of a 'sum over time' series, in which the developing population includes daughter cells of differing diminishing replicative ability and subsequent differentiation, cell death (programmed or otherwise), necrosis or senescence. Proliferation of CSCs depends in some way upon unidentified demand(s). The tempo of cancer proliferation is thought in part to be driven by active

proliferation of CSCs as they replenish or augment the transit-amplifying cell population. Prostate PC3 cell holoclones, characterized by morphology, epitope composition and behavior, have been identified as including CSCs, while meroclones and paraclones are included in their transit-amplifying populations that gradually undergo the changes previously described (26). Since, for some types of cancer, estimates of CSC numbers have been only a few percent of cells, it seems either that they need not replicate extensively if (i) supported by active transit cell proliferation, (ii) the number of CSC has been underestimated, or (iii) additional unidentified CSCs might exist, possibly to be evoked under specific niche conditions. If CSCs are not monolithic and a tumor contains CSC subtypes, their variation should influence response to therapy intentionally directed against one of them.

The ability of pancreatic and possibly other types of cancer to metastasize is apparently one of the last capacities to develop (10). Metastases from some treated tumors exhibit a long latency period, remaining indolent or even undetected for prolonged periods. Malignant melanoma provides an example in which metastases that are believed to have occurred before excision of the primary melanoma remain quiescent before actively proliferating years later (27). Additional genetic or epigenetic events and/or changes in the properties of the niche seem to be necessary for clinical recurrence.

If a sequence of genetic or epigenetic changes denoted as a series of related events A-> B-> C-> D-> E is required to yield a fully malignant pancreatic or other CSC able to metastasize, is it the case that interdiction of one of the intermediate stages prevents subsequent events from occurring and so precludes development of the terminal cell? In some types of solid cancer, do precursor forms as A-> B -> C represent 'benign' precursors of the evolving cancer? If a precursor stage is blocked, do alternate pathways develop? Genetic instability of malignantly transformed cells (28) and the epithelial to mesenchymal transition (12) contribute to the development of malignant properties.

Identifying mutual signaling between the transit-amplifying population and the CSC promoting their proliferation may provide sites for interdiction. Uncomplicated asymmetric proliferation (Appendix, Figure 1B) introduces a gradually declining ratio of CSCs to daughter cells, a potential form of feedback presumably also important in nonmalignant stem cell to daughter cell interactions. Further genetic changes or the rare reversion (de-differentiation) of a daughter cell to a CSC could underlie replacement in treated tumors of CSCs no longer susceptible to therapy, recreating in a different way what was considered as the original reason for the lack of therapeutic success, a potential form of an 'infinite regress.'

The mode of CSC proliferation, the fraction of tumor cells replicating and whether tumor size is reduced due to differentiation or cell loss should largely define the pace of cancer development over time, but their relative contributions varying in time and location remain unknown. When genetic or epigenetic changes with a low probability of occurrence are combined with a robust loss of transforming cells and an indolent proliferation rate, early detection by physical or x-rays examination is not possible. Three potential approaches for extending cancer latency periods, converting them to more chronic forms of illness, are: (i) identifying the reasons for long cancer latency periods, (ii) the mutual interactions between CSCs, their daughter cells and the niche they occupy, and (iii) whether intermediate but usually unidentified steps in malignant transformation provide sites for interdiction (8). Currently, surgical removal of suspect precursor or benign lesions and lifestyle changes related to diet, exercise and avoidance of known or suspected carcinogenic agents seem to represent the only practical means of potentially modifying the pace of pre-clinical development of most solid and possibly other types of cancer.

Acknowledgements

References

1 Sell S: History of cancer stem cells, in Regulatory Networks in Stem Cells. Rajasekhar VK and Vemuri MC (eds.). Humana Press, pp. 495-503, 2009.
2 Walker MR, Patel KK and Stappenbeck TS: The stem cell niche. J Path *217*: 169-180, 2009.
3 Cairns J: Mutation selection and the natural history of cancer. Nature *255*: 197-200, 1975.
4 Rosen JM and Jordan CT: The increasing complexity of the cancer stem cell paradigm. Science *324*: 1670-1673, 2009.
5 Demicheli R, Pratesi G and Foroni R: The exponential-Gompertzian tumor growth model: Data from six tumor cell lines *in vitro* and *in vivo*. Estimate of the transition point between exponential to Gompertzian growth and potential clinical implications. Tumori *77*: 189-195, 1991.
6 Till JE, McCulloch EA and Siminovitch L: A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. Proc Natl Acad Sci US *51*: 29-36, 1964.
7 Schatton T, Frank NY and Frank MH: Solid tumor stem cells – Implications for cancer therapy. In: Regulatory Networks in Stem Cells. Rajasekhar VK and Vemuri MC (eds.). Humana Press, pp. 527-543, 2009.
8 Folkman J: What is tumor dormancy? Can it be prolonged therapeutically? [David A. Karnofsky Memorial Lecture] [abstract]. Proc Am Soc Clin Oncol *15*: xix, 1996.
9 Jerry LM: The Epidemiology of Cancer. WHO Collaborative Centre, 1998. htpp://www.pitt.edu/-super1/lecture/lec0192/w007.htm.
10 Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JD, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B and Jacobuzlio-Donahue CA: Distant metastases occurs late during the genetic evolution of pancreatic cancer. Nature *467*: 1114-1120, 2010.

11 Stratton MP: Exploring the genomes of cancer cells: Progress and promise. Science *331*: 1553-1558, 2011.

12 Kalluri R and Weinberg RA: The basics of epithelial–mesenchyme transition. J Clin Invest *119*: 1420-1428, 2009.

13 Tannock IF: Tumor growth and cell kinetics. In: The Basic Science of Oncology, 2nd ed. Tannock IF and Hill RP (eds.). McGraw Hill, NY, pp. 154-177, 1992.

14 Rubinow SI: Introduction to Mathematical Biology, Chapter 1. Wiley, NY, 1975.

15 Hill RP and Perris R: 'Destemming' cancer stem cells. JNCI *99*: 1435-1441, 2007.

16 Houghton JM, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR and Wang TC: Gastric cancer originating from bone marrow-derived cells. Science *306*: 1568-1571, 2004.

17 Otto SP and Day T: A Biologist's Guide to Mathematical Modeling in Ecology and Evolution. Princeton Press, Princeton, NJ, pp. 567-607, 2007.

18 Agresti A: Categorical Data Analysis. Wiley, New York, 2002.

19 Wright DB and London K: Modern Regression Techniques Using R. Sage Publications, Thousand Oaks, CA, 2009.

20 Crawley MJ: Statistics: An Introduction Using R. Wiley, New York, 2005.

21 Venables WN, Smith DM and the R Development Core Team: An Introduction to R. Revolution Computing, Palo Alto, CA, 2009.

22 Norusis M: SPSS 17.0 Statistical Procedures Companion. Prentice Hall, Upper Saddle River, NJ, 2009.

23 Chen Z, Odstreil EA, Ti BP and McKnight SL: Restriction of DNA replication to the reductive phase of the metabolic cycle protects genomic integrity. Science *316*: 1916-1919, 2007.

24 Li L and Clevers H: Coexistence of quiescent and active adult stem cells in mammals. Science *327*: 542-545, 2010.

25 Dingli D, Traulsen A and Michor F: (A)symmetric stem cell replication and cancer. PLoS Comput Biol *3*: e53, 2007.

26 Li H, Chen X, Calhoun-Davis T, Claypool K and Tang DG: PC3 Human prostate carcinoma cell holoclones contain self-renewing tumor initiating cells. Cancer Res *68*: 1820-1825, 2008.

27 Yarnold JR and Cochrane JPS: Malignant melanoma: Significance of disease-free interval. Br J Radiol *53*: 1068-1070, 1980.

28 Hill RP: Tumor progression: potential role of unstable genomic changes. Cancer Metastasis Rev *9*: 137-147, 1990.