

The role of cell death in the growth of preneoplastic lesions: a Monte Carlo simulation model

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Abstract. A variety of experimental and clinical examples of preneoplasia demonstrate that regression of early lesions is common. This paper examines the hypothesis that early lesions operate under the identical growth kinetics of 'late' lesions (neoplasms), but that kinetic features favouring continuous growth in established lesions tend to favour extinction of lesions composed of small numbers of cells. Growth simulations of early lesions were produced using the Monte Carlo method, a technique demanding intensive computations. With the advent of powerful personal computers, this technique is now widely available to biologists. Simulating growth under conditions of cell loss similar to those observed in established tumours, the model predicts that the great majority of initiated cell clusters are expected to reach extinction within a few cell doubling times, and most early (promoted) lesions would not likely progress to the size of a clinically detectable lesion within the life span of the host organism. These Monte Carlo simulations provide a model of initiated cell growth consistent with the recently demonstrated role of early lesion cell death in the development of human lymphomas and in transgenic mice expressing the *bcl-2* oncogene. The model demonstrates that small increments in the intrinsic cell loss probability in even the earliest progenitors of malignancy can strongly influence the subsequent development of neoplasia from initiated foci.

In most animal models, the most likely outcome for preneoplastic lesions is regression, rather than progression. In the preneoplastic liver model (Solt, Medline & Farber 1977), hundreds of early liver lesions result in only one or a few established carcinomas. A variety of preneoplastic lesions in man, some with proven aneuploidy, have only a small chance of ever progressing to invasive cancer. These include cervical dysplasia, Barrett's oesophagus, oral leukoplakia, low-grade papillary tumours of bladder, lobular carcinoma *in situ* of breast, and borderline tumours of the ovary. In all these cases, progression to cancer is the exception, not the rule.

Two unproven and divergent theories addressing the phenomenon of early lesion regression are: **1** that early lesions are detected by a host immune surveillance system that destroys

preneoplastic cells identified by distinctive immunologic markers; **2** that early lesions do not have all the genetic changes necessary for sustained growth. We propose that the growth kinetics of early lesions may favour lesion regression, rather than early lesion growth. Recently we modelled tumour cell growth using an intrinsic cell death probability, and found that all observed human tumour growth could be modelled by cell death probabilities varying between 0.43 and 0.50 (Moore & Berman 1991, Berman & Moore 1992). In this model, tumours with cell death probabilities above 0.50 cannot increase their size over time, whereas tumours with cell death probabilities less than 0.43 grow faster than the fastest growing clinically observed tumours in man. In this study, we extend our model to simulations of small colonies of cells dividing with varying cell death rate probabilities. Simulations in this study demonstrate regression (extinction) of small colonies under the same growth kinetic parameters that were previously shown to produce continuous population expansion in large populations of cells (Berman & Moore 1992).

Experimental evidence for the role of cell death rates in carcinogenesis was recently provided by Korsmeyer and colleagues (Sentman *et al.* 1991, McDonnell & Korsmeyer 1991). This group has shown that the *bcl-2* oncogene, associated with malignant lymphoma in man, is an important inhibitor of programmed cell death. Transgenic mice with deregulated *bcl-2* in their germline develop polyclonal B cell hyperplasias leading to lymphomas. The increase in B cells in early lesions results from their extended survival rather than from increased proliferation. Other oncogenes (including the *myc* oncogene) and agents may exert their biological activity by blocking cell death (Evan *et al.* 1992, Rotello *et al.* 1991). Thus evidence is mounting that cell death in tumours is a biologically determined phenomenon and not the simple result of noxious microenvironmental conditions within the tumour.

MATERIALS AND METHODS

Monte Carlo cell growth models were programmed on an IBM PC/AT compatible computer (COMTEX, 80386 microprocessor, 25 MHz, 330 Mb Priam hard disk), using American National Standard MUMPS (MGlobal Inc., Houston, TX), and the public-domain File Manager (FileMan) database management system of the United States Department of Veterans Affairs (Davis 1987). We assume that each cell-cluster begins as a single cell that divides. At each new generation, each daughter cell may either divide or die. Death is determined by an inherited death probability, P , that is constant for the progenitor cell and all of its descendants. For the particular case in which there is no cell death, the number of cells at the n^{th} generation is 2^n .

This cell growth model is an example of a Galton-Watson branching process, for which the exact (analytic) solution for the probability of extinction is known in the limiting case of a cell population after an indefinite number of generations (Galton & Watson 1875, Jagers 1975, Kimmel & Axelrod 1991). In particular, the probability of extinction, q , for the descendants of a single progenitor (cell or person) is the smallest non-negative root of the equation $x = f(x)$, where $f(x)$ is the probability generating function, $f(x) = \sum (g_k x^k)$, from $k = 0$ to infinity, and g_k is the probability of having k children, constant among the progenitor and all descendants. For the present cell growth model, the cell death probability, P , applies equally and independently to both daughter cells resulting from each cell division, so that $g_0 = P^2$, $g_1 = 2P(1 - P)$, $g_2 = (1 - P)^2$, and $g_3, g_4, \dots = 0$. Solving for the smallest non-negative root of $f(x) = x$ by the quadratic formula yields the eventual proportion of extinct clusters after an indefinite generation number as 1 for $P \geq 0.50$, and $(P/(1 - P))^{2N}$ for $P < 0.50$, where N is the initial number of progenitor cells.

In a Monte Carlo simulation, a pseudorandom number generator is substituted for the probability value in the theoretical model (Cashwell & Everett 1959). In generation 0 there is a starting cell-cluster consisting of one or more premitotic cells (founder cluster), each of which is capable of at least one additional mitosis. The cell count thus refers only to those cells capable of an additional mitosis; this cell count may underestimate an observed cell count, which would include a variable number of postmitotic cells. Each premitotic cell in generation k divides to form two daughter cells in generation $k + 1$. For each daughter cell, a pseudorandom number, r , is selected independently from the uniform (equiprobable) density function over the unit interval. If $r < P$, then the cell is premitotic; if $r \geq P$, then the cell is incapable of further mitosis. The process continues to extinction of all premitotic cells, or to an arbitrary termination point (in this report, the 60th generation).

A sample output for 10 single cells, each with death probability 0.48 per generation, is shown in Table 1. In generation 0, there is a single, premitotic cell in each of the 10 clusters. Each premitotic cell divides once, producing two daughter cells apiece in generation 1. In clusters 2, 5, and 8, both daughter cells draw a pseudorandom number less than 0.48, and the cluster

Table 1. Ten Monte Carlo trials, one initial cell, death probability 0.48

Generation	Cluster no.									
	1	2	3	4	5	6	7	8	9	10
0	1	1	1	1	1	1	1	1	1	1
1	2	—	1	1	—	1	1	—	2	1
2	2	—	2	—	—	2	1	—	2	—
3	3	—	3	—	—	1	2	—	2	—
4	—	—	4	—	—	1	1	—	2	—
5	—	—	3	—	—	2	—	—	1	—
6	—	—	4	—	—	3	—	—	2	—
7	—	—	3	—	—	3	—	—	1	—
8	—	—	3	—	—	4	—	—	—	—
9	—	—	2	—	—	5	—	—	—	—
10	—	—	1	—	—	5	—	—	—	—
11	—	—	1	—	—	8	—	—	—	—
12	—	—	2	—	—	6	—	—	—	—
13	—	—	2	—	—	9	—	—	—	—
14	—	—	2	—	—	10	—	—	—	—
15	—	—	2	—	—	9	—	—	—	—
16	—	—	2	—	—	7	—	—	—	—
17	—	—	1	—	—	3	—	—	—	—
18	—	—	1	—	—	5	—	—	—	—
19	—	—	—	—	—	2	—	—	—	—
20	—	—	—	—	—	2	—	—	—	—
21	—	—	—	—	—	3	—	—	—	—
22	—	—	—	—	—	2	—	—	—	—
23	—	—	—	—	—	3	—	—	—	—
24	—	—	—	—	—	1	—	—	—	—
25	—	—	—	—	—	2	—	—	—	—
26	—	—	—	—	—	1	—	—	—	—
27	—	—	—	—	—	1	—	—	—	—
28	—	—	—	—	—	1	—	—	—	—
Extinction of all cell lines										
Execution complete										

ter becomes extinct. In clusters 3, 4, 6, 7 and 10, one daughter cell draws a pseudorandom number less than 0.48, while the other daughter cell draws a pseudorandom number at least 0.48, to yield one premitotic cell in generation 1. In clusters 1 and 9, both daughter cells draw a pseudorandom number at least 0.48, to yield two premitotic cells in generation 1. Clusters 4 and 10 become extinct in generation 2. Cluster 1 becomes extinct in generation 4. Cluster 7 becomes extinct in generation 5. Cluster 9 becomes extinct in generation 8. Cluster 3 becomes extinct in generation 19. Cluster 6 becomes extinct in generation 29.

In this study, we examined the behaviour of 100 initial cell clusters (Monte Carlo trials) at each of nine cell death probabilities (0.45, 0.46, 0.47, 0.48, 0.49, 0.50, 0.51, 0.52, and 0.53) and five initial cluster sizes ($N = 1, 5, 10, 25, 50$). Each cluster was arbitrarily terminated after generation 60. The mean and maximum sizes of clusters surviving at generation 60 was obtained. The Galton-Watson branching process was used to obtain the eventual proportion of surviving clusters after an indefinite number of generations.

RESULTS

Figure 1 shows the results of 100 Monte Carlo simulation trials after 60 generations, with an initial cluster size of one cell and death probability $P = 0.45$. Twenty initial clusters (20%) survived, ranging in size from 43 cells (surviving cluster 4) to 5396 cells (surviving cluster 15), and averaging 780 cells per surviving cluster. Eighty clusters (80%) became extinct. Tumours with a cell death probability of 0.45 and a cell cycle time of 1 day have a tumour doubling time of approximately 8 days (Moore & Berman 1991).

Table 2 shows the distribution of 100 cell clusters, each with initially 10 cells, at cell death probabilities 0.53, 0.52, 0.51, 0.50, 0.49, 0.48, 0.47, 0.46, or 0.45. After 60 generations, the 100 initial cell clusters with cell death probability 0.53 had three surviving clusters, the 100 initial cell clusters with cell death probability 0.52 had six surviving clusters, etc., as shown in

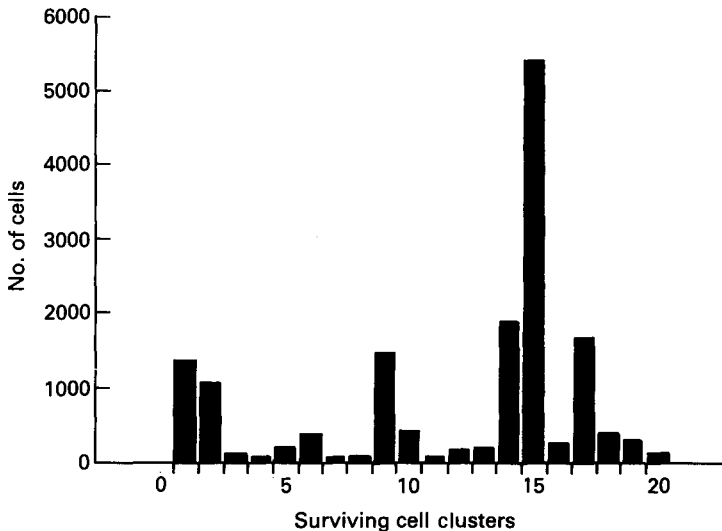


Figure 1. Results of 100 Monte Carlo simulation trials after 60 generations, with an initial cluster size of one cell and cell death probability, $P = 0.45$. Twenty clusters survived, ranging in size from 43 cells (surviving cluster 4) to 5396 cells (surviving cluster 15), and averaging 780 cells per surviving cluster.

Table 2. Distribution of clusters in 100 Monte Carlo trials with 10-cell starting clusters, and different cell death probabilities

Probability of cell death per cell per generation	% clusters surviving to 60 generations	Eventual % clusters surviving (Galton-Watson)	Largest surviving cluster	Average size of surviving clusters, no. of cells
0.53	3	0	30	15
0.52	6	0	43	16
0.51	8	0	37	10
0.50	29	0	59	19
0.49	47	55	197	40
0.48	79	80	528	107
0.47	88	91	1283	265
0.46	90	96	3850	925
0.45	99	98	9117	2001

column 2. Column 3 of Table 2 shows the eventual proportion of surviving clusters after an indefinite generation number, as predicted by the Galton-Watson branching process. For cell death probability $P \geq 0.50$, the eventual proportion of surviving clusters is 0; for lower cell death probabilities, the clusters surviving after 60 generations and those surviving after an indefinite number of generations are very similar.

As shown in columns 4 and 5 of Table 2, among the three surviving clusters after 60 generations at cell death probability 0.53, the largest surviving cluster had 30 cells, and the average cluster size was 15 cells. Among the 100 initial cell clusters with cell death probability 0.45, after 60 generations there were 99 surviving clusters, in which the largest surviving cluster had 9117 cells, the average cluster size was 2001 cells; and the eventual number of surviving clusters was 98. Clearly the four measures of cluster survival, namely per cent of surviving clusters after 60 generations, the eventual per cent of surviving clusters, the size of largest surviving cluster after 60 generations, and the mean surviving cluster size, all have an inverse relationship with cell death probability.

Table 3 shows the distribution of 100 cell clusters, each with initial cluster sizes of one, five, 10, 25, or 50 cells, at cell death probabilities 0.53, 0.52, 0.51, 0.50, 0.49, 0.48, 0.47, 0.46, or 0.45. After 60 generations, the 100 initial cell clusters with cell death probability 0.53 and initial cluster size 1 had one surviving cluster containing two cells, whereas the 100 initial cell clusters with cell death probability 0.45 and initial cluster size 50 had 100 surviving clusters with a maximum cluster size of 28 208 cells. Eventually after an indefinite number of generations, the 100 initial cell clusters with cell death probability 0.53 and initial cluster size 1 had no surviving clusters, whereas the 100 initial cell clusters with cell death probability 0.45 and initial cluster size 50 had 100 surviving clusters. The number of surviving clusters and maximum cluster size after 60 or an indefinite number of generations are inversely correlated with cell death probability and directly correlated with initial cluster size.

DISCUSSION

Two popular hypotheses address the phenomenon of early lesion regression: **1** that early lesions are caught by a host immune surveillance system that detects and destroys preneoplastic cells identified by distinctive immunological markers; **2** that early lesions do not have all the

Table 3. Survival of clusters, varying initial cluster sizes and cell death probabilities

Probability of cell death per cell per generation	Total surviving clusters at 60 generations [total eventual surviving clusters] (size of largest cluster)*				
	Initial cluster size				
	1	5	10	25	50
0.53	1 [0] (2)	3 [0] (28)	3 [0] (30)	7 [0] (17)	13 [0] (21)
0.52	0 [0] (0)	1 [0] (6)	6 [0] (43)	23 [0] (23)	33 [0] (37)
0.51	1 [0] (14)	9 [0] (22)	8 [0] (37)	39 [0] (52)	67 [0] (94)
0.50	4 [0] (38)	11 [0] (24)	29 [0] (59)	68 [0] (114)	88 [0] (157)
0.49	8 [8] (145)	34 [32] (184)	47 [55] (197)	87 [86] (275)	93 [100] (678)
0.48	16 [15] (256)	45 [55] (228)	79 [80] (528)	93 [98] (930)	100 [100] (1638)
0.47	15 [21] (556)	64 [70] (1105)	88 [91] (1283)	99 [100] (2780)	99 [100] (4724)
0.46	23 [27] (800)	59 [80] (2508)	90 [96] (3850)	99 [100] (6252)	100 [100] (11182)
0.45	20 [33] (5396)	80 [87] (6245)	99 [98] (9117)	99 [100] (15269)	100 [100] (28208)

* The first number is the number of original 100 clusters that survived 60 generations. The number in square brackets [] is the number of original 100 clusters that eventually survived (Galton-Watson model). The number in parentheses () is the number of cells in the largest cluster at generation 60.

genetic lesions necessary for sustained growth. Both these hypotheses have drawbacks. The immune surveillance mechanism for early lesion regression fails to explain the lack of heightened cancer susceptibility in immune deficient mice (nude mice). In man, immune suppression is not associated with increased risk of malignancy for most tumours. Rather, immune suppressed patients seem to develop tumours whose aetiology is directly dependent on the immune deficient state for growth (i.e., lymphomas and virally-induced neoplasms). The second argument, that early lesions must obtain additional mutations in order to grow as fully developed neoplasms, implies that the additional genetic changes observed in neoplastic development (such as oncogene acquisition or suppressor gene loss) involve growth kinetics, an unproven hypothesis. It is completely feasible that additional and necessary genetic alterations attained during neoplastic development may relate to phenotypic properties independent from growth kinetics (e.g. properties related to invasiveness and not to cell proliferation).

Evidence supporting similar growth kinetics for early lesions and for late lesions is provided by the work of Collins and colleagues, who compared the time needed for tumours to evolve with the time needed for tumours to recur after unsuccessful treatment (Collins, Loeffler & Tivey 1956, Collins 1958). For example, gestational chorio-carcinoma is almost always detected by the 13th month following conception. When a recurrence appears, it almost always appears within 13 months of tumour treatment. Similar results are observed in studies of Wilms' tumours and of Burkitt's tumours (Bagshawe 1976, Iverson *et al.* 1974). In the case of solid tumours in adults, recurrences may appear decades after treatment of the tumours, but these tumours also seem to have a very long evolution. These studies would suggest that the growth kinetics of an initiated cell or a cell in an early preneoplastic lesion or a cell in a developed tumour all have the same growth kinetics.

Laird (1969) has demonstrated that cell division in many tumours is accompanied by high rates of tumour cell death. Most tumours consist of a large proportion of cells that are not capable of further division, typically in the range of 20–70% (Schiffer 1987). Many tumours have a growth fraction of only 25% and a cell loss factor of 70% or more (Schiffer 1987). Even

normal tissue has a high rate of cell loss. In skin and gut, under conditions of homeostasis, each dividing cell produces two daughter cells, one that can divide and another that is post-mitotic (i.e. moribund). The destiny of the post-mitotic cell is to be sloughed (in the case of skin) or extruded (in the case of the gut). Normal tissue is apparently balanced by a 50% probability of cell death (end-stage differentiation) for cell divisions. In normal tissue that is not undergoing net growth (e.g. epidermis), a near 50% probability of cell death (via end-stage differentiation) is the rule. For example, when a basal cell of skin divides, it produces a differentiated skin cell (to replace the sloughed cell of the stratum corneum) and another basal cell (to replace itself). This is not to say that whenever a basal cell divides, the determination of which daughter cell will live (replicate again) and which daughter cell will die (become postmitotic) is determined *in vivo* on a probabilistic basis. We presume that a biological mechanism exists to control the proliferative destiny of the daughter cells. However, since we know that on average one cell dies for each cell that lives, we can model normal epidermal growth in a probabilistic model wherein each cell has a near 50% chance of dying in any cell cycle. Furthermore, the rate of cell death is static only under the condition of no net growth (the normal condition of skin). During wound repair, for example, the rate of cell growth must at least temporarily exceed the rate of cell death. Wound repair would be modelled with a cell death probability less than 50%.

The likelihood of a cell entering a non-dividing state, although presumably determined by specific biological mechanisms, can be modelled probabilistically, since tumours as a whole seem to maintain characteristic population cell loss rates (Schiffer 1987). We performed computer simulations of the growth of clusters of cells, each cell of the cluster growing with the same probability of cell death as all other cells in the cluster. Simulations were performed to determine whether the extinction of cell clusters initially containing only a few cells might result under the same growth kinetic conditions that favour continuous growth of initially large cell clusters. Additional Monte Carlo simulations were performed for model populations of initiated cells having different constant cell death probabilities. Repeated Monte Carlo simulations using the same set of initial parameters yield different outcomes (a range of possible results), permitting analysis of potentially unexpected trends and the detection of unusual events.

We have shown previously that Monte Carlo modelling of tumour doubling times at various cell death rates can model the observed range of human tumour doubling times (Moore & Berman 1991). A cell doubling time of a little over 6 days is modelled by a cell death probability of 0.44 and a cell cycle time of 1 day. A cell doubling time of about 45 days is modelled by a cell death probability of 0.49 and a cell cycle time of 1 day.

A Monte Carlo simulation, applied to a probabilistic problem, does not obtain an analytic solution, but rather substitutes a pseudorandom number generator for the probability value in the theoretical distribution. Then a series of computational experiments are performed. If an analytic solution were obtained for cell death probability P , then the solution at the n th generation would have 2^n possible outcomes. It is apparent that the analytic solution is too unwieldy to examine the model's average behaviour. In our opinion, simulation models have some important benefits compared to analytic models incorporating cell death probabilities (Takahashi 1968, Goldie 1989, Kimmel & Axelrod 1991) because they can be used to describe the behaviour of individual components of a system, whereas analytic systems often deal with the aggregate system behaviour (Widman & Lopar 1990).

Repeated Monte Carlo simulations produce a variety of possible outcomes for a single set of initial conditions. In this case, Monte Carlo simulation demonstrated how early lesions may reach extinction. Simulations demonstrated cell growth trends that would not have been predicted on a purely intuitive basis. This is particularly true in the case of the maximal regressing

cluster size. Among all outcomes, regardless of the intrinsic cell death probability, once a cluster reaches a size of about 50 cells, it does not regress to 0 (Berman & Moore 1992). In a deterministic model that allows growth among fractional cells, this finding could not have been anticipated. In our model, small differences (on the order of 1%) in the cell death probability account for large differences in the number of surviving clusters at the 60th generation. Although this paper does not specifically address the biological roles of initiation and promotion, one can speculate that promotion may exert its biological effect through a downward adjustment of the cell death rate of initiated cells, leading to rapid expansion of initiated clusters.

It is important to add that the rate of cell proliferation does not change the number of cell clusters that reach extinction. The rate of proliferation only changes the speed with which clusters become extinct. The model serves to demonstrate that simulating cell growth by conditions assumed in this study often results in population extinction. Like all models, ours does not provide a proof of biological mechanism. It does allow us to see biological possibilities that are not intuitively obvious or, in the case of Monte Carlo models, are not readily predicted by more traditional analytic methods.

The fact that a population of persons can exhibit exponential growth was appreciated by Leonhard Euler in the mid-18th century and later by Thomas R. Malthus. Despite this rapid growth in population size, Malthus noted, almost in passing, that all the lineages of a majority of individual progenitors (specifically, bourgeois families in Berne, Switzerland) tended to die out over time (Jagers 1975). Galton & Watson (1875) formulated the problem as a probability generating function, $f(x) = \sum(g_k x^k)$, from $k = 0$ to infinity, and g_k is the probability of having k children, constant among the progenitor and all descendants. Then the probability of one progenitor's family becoming extinct is the smallest non-negative root of the equation, $x = f(x)$. As shown in Table 3, the probability of eventual survival is quite close to our Monte Carlo estimations after 60 generations. Theoretical values are not subject to random fluctuations inherent in Monte Carlo methods, but Monte Carlo methods offer the comparative advantage of simplicity in setting up the calculations for microcomputers, and thus in rapidly obtaining an approximate idea of the final, numerical values. Furthermore, Monte Carlo methods carried out on a computer can be interrupted at various steps in the calculation, giving the investigator a sense of the flow of cell growth and death for individual cases.

In previous studies by the authors, the growth of neoplasms was modelled by Monte Carlo simulations. The present study extends the results obtained for established lesions to preneoplastic lesions. We demonstrate that small cell populations may tend to regress under the same growth kinetics that result in rapid growth of large populations of cells (established neoplasms). These findings provide a plausible explanation for the regression of preneoplastic lesions (including the extinction of initiated cells) that does not assume the existence of biological properties beyond those already observed in experimental and human neoplastic development. These simulations also quantitatively model the effect on proliferating populations exerted by modulators of cell death (such as the *bcl-2* and *myc* oncogenes).

REFERENCES

- BAGSHAWE KD. (1976) Risk and prognostic factors in trophoblastic neoplasia. *Cancer*, **38**, 1373.
 BERMAN JJ, MOORE GW. (1992) Spontaneous regression of residual tumor burden: prediction by Monte Carlo simulation. *Analyt. Cell. Pathol.* In press.
 CASHWELL ED, EVERETT CJ. (1959) *A Practical Manual on the Monte Carlo Method for Random Walk Problems*. New York: Pergamon Press.
 COLLINS VP. (1958) The treatment of Wilms' tumor. *Cancer*, **11**, 89.

- COLLINS VP, LOEFFLER RK, TIVEY H. (1956) Observations on growth rates of human tumours. *Am. J. Roentgenol.* **76**, 988.
- DAVIS RG. (1987) *FileMan: A User Manual*. Bethesda, Maryland: National Association of VA Physicians.
- EVAN GI, WYLLIE AH, GILBERT CS *et al.* (1992) Induction of apoptosis in fibroblasts by c-myc protein. *Cell*, **69**, 119.
- GALTON F, WATSON HW. (1875) On the probability of extinction of families. *J. R. Anthropol. Inst.*, **4**, 138.
- GOLDIE JH. (1989) Mathematical model of drug resistance and chemotherapy effects. *Cancer Treat. Res.* **48**, 13.
- IVERSON OH, IVERSON U, ZIEGLER JL, BLUMING A. (1974) Cell kinetics in Burkitt's lymphoma. *Eur. J. Cancer*, **10**, 155.
- JAGERS P. (1975) *Branching Processes with Biological Applications*. London: John Wiley & Sons, 1.
- KIMMEL M, AXELROD DE. (1991) Unequal cell division, growth regulation and colony size of mammalian cells: a mathematical model and analysis of experimental data. *J. Theor. Biol.* **153**, 157.
- LAIRD AK. (1969) Dynamics of growth in tumours and in normal organisms. *Natl. Cancer Inst. Monogr.* **30**, 15.
- MCDONNELL TJ, KORSMEYER SJ. (1991) Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). *Nature*, **349**, 254.
- MOORE GW, BERMAN JJ. (1991) Cell growth simulations that predict polyclonal origins for 'monoclonal' tumours. *Cancer Letts.* **60**, 113.
- ROTELLO RJ, LIEBERMAN RC, PURCHIO AF, GERSCHENSON LE. (1991) Coordinated regulation of apoptosis and cell proliferation by transforming growth factor beta 1 in cultured uterine epithelial cells. *Proc. Natl. Acad. Sci. USA*, **88**, 3412.
- SCHIFFER LM. (1987) Cellular proliferation in tumor and in normal tissues. In: Perez CA, Brady LW, eds. *Principles and Practice of Radiation Oncology*. Philadelphia: Lippincott, 56.
- SENTMAN CL, SHUTTER JR, HOCKENBERRY D, KANAGAWA O, KORSMEYER SJ. (1991) Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell*, **67**, 879.
- SOLT DB, MEDLINE A, FARBER E. (1977) Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *Am. J. Pathol.* **88**, 595.
- TAKAHASHI M. (1968) Theoretical basis for cell cycle analysis II. Further studies on labelled mitosis wave method. *J. Theoret. Biol.* **18**, 195.
- WIDMAN LW, LOPARO KA. (1990) Artificial intelligence, simulation, and modeling. *Interfaces* **20**, 48.