Chemoprevention of colon cancer: Advantage of intermittent pulse treatment schedules quantified by computer simulation of human colon crypts

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Chemoprevention of colon cancer: Advantage of intermittent pulse treatment schedules quantified by computer simulation of human colon crypts

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Running head: Chemoprevention of colon cancer
Abstract

Background: Intermittent treatment schedules have been proposed to improve the tolerance of drugs for cancer chemoprevention. However, determining a maximum tolerated dose, and the extent of the improvement, has been challenging experimentally and clinically. Methods: In order to determine the quantitative advantage of intermittent pulse treatment schedules for the chemoprevention of colon cancer we have used a computer model of human colon crypts calibrated with measurements of human biopsy specimens. In simulations, crypts were treated with an agent that increases the probability that cells, both normal and mutant, would be removed at the top of the crypt. Sulindac, which increases apoptosis at the lumen surface, is such an agent. The effect of intermittent pulse drug treatment schedules were compared with constant drug treatment schedules. Results: Crypts treated with intermittent pulse schedules have three times the maximum tolerated dose than crypts treated with constant schedules, and have a 10 year delay in the appearance of adenomas. Conclusions: Intermittent treatment schedules have previously been proposed for chemoprevention. Here computer simulations have quantified the effect on human colon crypts of intermittent treatment schedules and constant treatment schedules of a chemotherapeutic drug. Intermittent pulses have an advantage, they allow an increased maximum tolerated dose, and result in an increased chemoprevention by delay.

Introduction
Effective chemoprevention of cancer requires choices of, \( (i) \) an agent(s) that is effective with little or no undesirable side effects, \( (ii) \) patients who would benefit from the exposure to the agent, and \( (iii) \) dose intensity and dose schedules that maximize the prevention effect and minimize the negative effects. This report concerns dose intensity and dose schedules. It provides computer simulation results that indicate the quantitative advantage of intermittent pulse dose schedules for the prevention by delay of colon cancer.

In the normal colon crypt, cells are born at the bottom, move up the crypt, and are removed at the top. An early stage of colon cancer may develop if a mutant cell spontaneously arises in a crypt but is not removed before it proliferates, establishes a population of abnormal cells that fill the crypt, and forms an adenoma. Our working hypothesis is that enhancing the movement of cells up and out of the crypt could increase the probability that mutant cells would be eliminated before they could establish a population of mutant cells. This could result in “chemoprevention by delay” [1].

The problem is to determine treatments that would remove mutant cells from the crypt while retaining the number of normal cells. We propose a chemoprevention strategy that is not aimed at killing spontaneously occurring mutant cells, but rather, is aimed at altering cell dynamics in the crypt. Specifically we intend to alter the efficiency by which a crypt removes all cells, both mutant and normal. The reduced number of normal cells could then be regenerated by quiescent stem cells that respond to the reduced number of cells and are activated to divide and produce more normal cells. This would restore the total number of normal cells
in the crypt. The challenge is to design a chemoprevention strategy that would reduce, but not eliminate, all cells in the crypt.

Some previous attempts at designing chemoprevention strategies have had limited success because low doses of agents are insufficiently effective, and higher doses have unacceptable side effects [2]. Paracelsus, considered the father of toxicology, in the 16th century summarized this situation by stating “The dose is the poison”. One suggestion to overcome the problem of toxicity of high doses is to use short-term intermittent dose schedules [3-5]. Intermittent doses schedules for chemoprevention have been reported for cell culture models [6] and animal models [7-10]. In order to translate intermittent dose schedules to humans it would be useful to have a system that could quantitatively predict the highest tolerated doses that would be effective, and the degree of the advantage of intermittent dose schedules compared to constant dose schedules.

We previously developed a computer model of cell dynamics in human colon crypts and used it to investigated chemotherapy schedules. Simulation results indicated that intermittent doses could result in the removal of mutant cells that had already filled the crypt and formed an adenoma, while allowing the recovery of normal crypt function [11]. That model has now been enhanced to allow investigation of chemoprevention treatment schedules that would delay the formation of adenomas by spontaneously occurring mutant cells.

Here we report on simulation results using a computer model of human colon crypts and use it to answer the following questions about intermittent pulse dose scheduling for chemoprevention of colon cancer:
1. What are the maximum tolerated doses for constant treatment and for intermittent pulse treatment schedules?

2. What is the quantitative advantage of intermittent pulse treatment schedules compared to constant treatment schedules, in preventing the appearance of adenomas in colon crypts, e.g. cancer chemoprevention by delay?

**Methods**

**Calibrated computer model of human colon crypts**

A virtual crypt model was produced with the NetLogo v.4.1.3 application. NetLogo is a multi-agent programmable modeling environment. It is authored by Uri Wilensky and developed at The Center for Connected Learning (CCL) and Computer-Based Modeling. It is a multi-platform (Mac or Windows) open-source application available to download at http://ccl.northwestern.edu/netlogo/.

We previously described an agent-based model of normal human colon crypts [11]. The model was calibrated with cell numbers measured in biopsy specimens. A slide containing sections from a biopsy of the sigmoid colon of a normal patient was stained with antibody MIB-1 to the G1 proliferation antigen Ki-67, and counterstained with hematoxylin. The number of cells appearing in the two-dimensional image of a cross section of each of 49 crypts were counted and used to calculate the number of cells in a three-dimensional crypt. Cells stained positively for the Ki-67 proliferation antigen were considered to be proliferating cells. Unstained cells at the bottom of the crypt were considered to be quiescent stem cells, and unstained cells near the top of the crypt were considered to be
differentiated cells. The mean and standard deviation of each cell type was the following: quiescent stem cells, 35.7 +/- 36.3, proliferating cells, 623.9 +/- 234.1, differentiated cells, 2427.8 +/- 504.4. Details of the previous version of the virtual crypt model, including graphical user interface, computer code, and comments were included. Details of image acquisition, measurements by image analysis, and determination of reliability of measurements were described in detail in file 5 of reference 11.

The model assumed that the probability of a cell’s proliferating along the crypt axis was determined, in part, by its position in a divide gradient in the microenvironment along the crypt axis, with the probability higher at the bottom than at the top. Stem cells at the bottom are in a quiescent niche. The probability of cell’s dying was determined, in part, by a cell’s position in a die gradient, higher at the top than at the bottom of the crypt. Model parameter values of the extracellular gradients were determined that reproduced the number and variation in each cell type measured in normal human colon crypts. Each simulation run was initiated with the same gradient values. The stochastic variation between runs resulted from, in addition to each cell’s position in the gradients, a different random number between 0 and 1.

The behavior of the model was verified as reproducing three features of biological crypts that had been previously observed, i.e. induction of adenomas by mutations occurring at the top or bottom of the crypts [13], monoclonal conversion by neutral drift [12], and robust recovery from perturbation by exposure to a cytotoxic agent [14]. Simulations indicated that mutants could be eliminated, while
sparing normal crypt function, if a cytotoxic chemotherapy treatment was intermittent with a duration of 3 ticks and interval between doses of 24 ticks. One computer “tick” corresponds to approximately 4.5 hours human time.

A new enhanced version of the model used here, adds the capability to simulate chemoprevention in addition to chemotherapy. The new model simulates chemoprevention by an agent that increases the probability that a cell, normal or mutant, will be removed at the top of the crypt. This is implemented by increasing the value of the parameter Crypt Die Maximum (CptDieMax). The model program “Colon Crypt Model 031215.nlogo”, 131 KB, is available to download at http://dx.doi.org/doi:10.7282/T3TQ638W. The model program runs on the open-source multi-platform NetLogo application, version 4.1.3, available to download at http://ccl.northwestern.edu/netlogo/.

Specimens

D.E.A. obtained coded de-identified slides containing sections from biopsies of the sigmoid colon of normal patients enrolled in a clinical research study of Dr. Steven Shiff, Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey. Individualized information about the identity of the subjects and specific clinical information were not made available to D.E.A. Dr. Shiff received approval Reference Number 4611 from the Institutional Review Board for Research Study Involving Human Subjects. The approved protocol included informed consent of participants.
**Statistical analysis**

Data generated by the Behavior Space tool of NetLogo was imported into JMP 10 or JMP Pro 11 (SAS Institute, Cary, NC 27513, USA) to generate plots and for statistical analysis. The Shapiro-Wilk W test was used to determine if distributions were normally distributed. The Wilcoxon test was used to determine the significance within and between groups of non-normal distributions. Simulations and analysis were done on an iMac computer, OS 10.9.5.

**Results**

**Exploiting cell dynamics in normal crypts**

Quiescent stem cells at the bottom of a normal crypt may become active stem cells and divide. The progeny cells move up the crypt and continue to divide and to differentiate. The probability of cell division decreases as cells move up the crypt, and the probability of cell differentiation increases as cells move up the crypt. At the top of the crypt the terminally differentiated cells are removed [12].

If mutant cells appear they may also move up the crypt and be removed. However, if mutant cells have an increased rate of proliferation they may give rise to a population of mutant cells that fill the crypt and form an early stage adenoma before the mutant cells are removed. We propose a form of chemoprevention that alters crypts so that mutant cells are more efficiently removed before the mutant population can form an adenoma.

The chemoprevention treatment is targeted at the crypt rather than at the mutant cells. The dose is designed to increase the probability that any cell, mutant
or normal, will be removed at the top of the crypt. The treatment utilizes the highest dose that a normal crypt can tolerate when given in intermittent pulses. Intermittent treatment allows the crypt to recover between doses.

**Computer model of human colon crypts for chemoprevention studies**

A new version of the colon crypt model allows the user to simulate intermittent chemoprevention. The investigator can input, at the user interface, parameter values for chemoprevention including dose intensity, dose duration, and dose interval. A range of values can be input at the same time in order to do parameter sweeping. The quantitative output is available in spreadsheet format for statistical analysis. The computer program, including interface, code, and detailed information is available as open source software at http://dx.doi.org/doi:10.7282/T3TQ638W.

In this report the new version of the model is used to investigate chemoprevention schedules. We have asked if intermittent treatment schedules could be superior to constant treatment schedules, and if so, what is the quantitative advantage to intermittent treatment schedules for chemoprevention?

**Crypts tolerate higher doses when treated intermittently than constantly**

In order to enhance the crypt’s ability to move mutant cells out of the crypt, we simulated the effect of a drug that would increase the probability that any cell, normal or mutant, would die and be removed toward the top of the crypt. This might be achieved by treatment with a drug that would induce apoptosis near the top of the crypt, for instance sulindac [15].
The model parameter that simulates an increased probability that a cell would die toward the top of the crypt is Crypt Die Maximum (CptDieMax). Three treatment schedules were simulated, a constant value of CptDieMax = 0.2, an increased constant value of CptDieMax = 0.5, and an intermittent pulse of CptDieMax = 1.4 with a duration = 3 ticks and interval between pulses = 24 ticks (Figure 1, Top). The value of CptDieMax = 0.2 was previously determined to reproduce the average number of cells per crypt measured in untreated normal human biopsy specimens, and is therefore referred to as no treatment. The values of duration = 3 ticks and interval = 24 ticks are the same as previously reported to be effective for chemotherapy [11].

The effect of these different treatment schedules on the number of cells per crypt is seen in Figure 1, Bottom. Crypts exposed to constant CptDieMax = 0.2 maintain a quasi-stationary number of cells per crypt that reproduces the average measured in biological crypts. Crypts exposed to constant CptDieMax = 0.5 maintain a smaller number of cells per crypt. Crypts exposed to an intermittent pulse of CptDieMax = 1.4, have a number of cells that oscillate between 2470 cells per crypt and minimum of about 1500 cells per crypt. The decrease in the number of cells per crypt is due to the increase in the value of the die gradient at the top of the crypt; the increase and recovery in the number of cells when the value of CptDieMax returns to 0.2, is due to the quiescent stem cells becoming activated to divide which restores the number of cells in a normal crypt. A similar robust response to perturbation and recovery has been reported for biological crypts [14] and for the computer model [11]. Crypts recovering from perturbation would be expected to remain functional.
The maximum treatment (CptDieMax) that a crypt can tolerate is shown in Figure 2. A crypt exposed to a constant treatment in the range of CptDieMax = 0.2 to 0.5 will survive, but a crypt exposed to a constant treatment of greater than 0.5 will collapse. In contrast, a crypt exposed to an intermittent pulse can survive a much greater treatment, up to CptDieMax = 1.4 Therefore, crypts tolerate higher doses when exposed to intermittent pulse treatment than constant treatment.

The effect of treating crypts with constant and intermittent pulse schedules can be seen in the movie available at http://dx.doi.org/doi:10.7282/T3RN39JH. Crypts treated with a high constant dose will collapse, but crypts treated with an equal high intermittent pulse will oscillate in size but not collapse. If mutants appear in crypts treated with a high intermittent pulse schedule, the mutants will be flushed out of the crypt and the crypt will recover.

**Mutants are delayed in forming adenomas when crypts are treated intermittently**

The previous section indicated the effect of different treatment schedules on the number of cells per crypt, before the spontaneous appearance of mutant cells. In this section the effect of simulating different treatment schedules on the number of mutants per crypt as a function of time is described.

In initial simulations, mutants were set to appear at the proportion of 0.0001 per cell per tick anywhere in the crypt. Other values of the proportion were used in later simulations, and results are given in Table 2. In these initial simulations mutants were assigned a probability of dividing of 1.16x that of normal cells at the
same position in the divide gradient along the crypt axis. Other values of the probability of dividing were used in later simulations, and results are given in Table 2.

Complete prevention would be accomplished if all spontaneously occurring mutant cells, and their mutant progeny, were removed before they could fill a crypt and form an adenoma. Prevention by delay [1] could be accomplished if most mutants are removed, but that rarely, after a long time, one mutant could produce a population of mutant progeny that fills a crypt. In other words, there would be a delay in time when an adenoma would form compared to no treatment.

The effect of different treatment schedules on the time at which spontaneous mutants survive, produce mutant progeny and fill a crypt, is shown in Figure 3, Left. The three treatment schedules are the following: no treatment CptDieMax = 0.2, constant treatment at the maximum tolerated CptDieMax = 0.5, and intermittent pulse treatment CptDieMax = 1.4. Each panel is an example of a single simulation run, the results of multiple runs will be described later. With no treatment mutant progeny fill the crypt after about 600 ticks, with constant treatment the appearance of mutant progeny that fill the crypt is delayed to about 4,000 ticks, with intermittent pulse treatment the appearance of mutant progeny that fill the crypt is much further delayed, to greater than 20,000 ticks.

A crypt that is filled with mutant cells and continues to increase in the number of cells can be considered to be adenoma. The effect of the three treatment schedules on the total number of cells per crypt is shown in Figure 3, Right. Constant treatment delays the time to form an adenoma compared to no treatment, and
intermittent pulse treatment delays the time to form an adenoma even more. For crypts treated with intermittent pulses, the number of cells per crypt varies with quasi-stationary stochastic dynamics (thick black tracing), until it increases without bounds and forms an adenoma beyond 20,000 ticks.

The panels in Figure 3 indicated that an intermittent pulse treatment schedule can delay the formation of an adenoma. However, each of these panels showed the results of only one example simulation run. Since the time of appearance of spontaneous mutants is random, it would be expected that the time to form an adenoma would be different in each simulation run. In order to determine the reliability of the conclusion that intermittent pulse treatment delays the formation of adenomas, and to determine the magnitude of the delay, a pair of 50 different simulations were run for each treatment. The results are shown in Figure 4. Constant treatment delays the time to form an adenoma compared to no treatment, and intermittent pulse treatment delays the time to form an adenoma even more.

The numerical values are shown in Table 1. The times to form an adenoma in pairs of runs with the same treatment schedule are not significantly different, but the times to form an adenoma with different treatment schedules are significantly different. The magnitude of the delay in formation of an adenoma was calculated using the value 1 computer tick = 4.5 human hours [11]. With no treatment the median time to form an adenoma would be expected to be about 0.3 years, with a constant treatment schedule about 1.5 years, and with an intermittent pulse treatment schedule about 10 years. Therefore, the intermittent pulse treatment
schedule has an advantage compared to the maximum tolerated constant treatment schedule by producing a dramatic delay in the formation of an adenoma.

The estimated delay produced with intermittent pulse treatment was calculated with a specific value of the proportion of cells that would be mutated at each tick, and a specific value for the difference between the mutant and the normal cells in the probability that each cell type would divide at the same position in the divide gradient along the crypt. However, spontaneous mutations might be expected to occur at different proportions, and the mutant cells might have different probabilities of dividing. In order to determine the generality of the conclusions about the advantage of intermittent pulse treatment schedule, simulations were conducted with other values than the reference values, Table 2. The time to form an adenoma with constant treatment schedule is compared to intermittent pulse treatment schedule, for other example values of Mutant Divide Difference, and other values of the Proportion of mutants that would form per tick. In each case the time to form an adenoma is greater for intermittent pulse treatment schedules than for constant treatment schedules. Therefore, the advantage of intermittent pulse treatment can be generalized for mutants dividing at different rates and occurring with different probabilities.

**Discussion**

We have described a mode of primary chemoprevention in which an altered crypt is made “inhospitable” to spontaneously occurring mutant cells by intermittent pulse treatment schedules. In contrast to secondary chemoprevention that is aimed at
mutant cells that already exist in an adenoma, the primary chemoprevention strategy suggested here is not aimed at the mutant cells but rather is aimed at the crypt even before the mutant cells appear. In the altered crypt, all cells, including the mutant cells, have an increased probability of being removed at the top of the crypt. Normal cells are regenerated by the stem cells and proliferating cells near the bottom of the crypt. Most mutant cells that appear will be removed before they divide and can establish a stable population of mutant progeny. However, with a low probability, after a long time, a rare mutant cell may survive to establish a stable population of mutant progeny that fill the crypt and form an adenoma. This is a stochastic process that results in chemoprevention by delay [1]. The strategy is effective against mutants which may arise at different places in the crypt (as was simulated here), at different times (Figure 3), have different probabilities of occurring (Table 2), and have different proliferation rates (Table 2).

A video (MP4-1 format, with audio) showing the advantage of intermittent pulse schedules compared to constant schedules for the chemoprevention of colon cancer, is available to download at http://dx.doi.org/doi:10.7282/T3RN39JH.

The advantage of intermittent pulse treatment schedules over constant treatment schedules, in delaying the time to form an adenoma, does not depend on a unique set of parameter values. The reference value used in the initial simulations for mutation probability would result in about one mutant cell per crypt per day. Other mutation probabilities, with a range of two orders of magnitude, also show an advantage of intermittent pulse treatment schedules (Table 2). This range of mutation probabilities per crypt per day encompasses the value reported for
somatic cell mutation rates per cell division [16]. The advantage of intermittent pulse treatment schedules is also obtained for a range of mutants with different probabilities of dividing compared to normal cells (Table 2), although over a smaller range. Since the mutations were induced at random at any location in the crypt, the advantage does not depend upon the location of initiating mutation.

Other parameter values were determined by parameter sweeping. The duration of the pulse was previously determined as the minimum that intermittent chemotherapy would remove all established mutants from a crypt [11]. The interval between pulses was determined by the time that the cell numbers returned to normal (Figure 1). The parameter values for the maximum tolerated dose for chemoprevention by intermittent pulse schedules and constant schedules were determined in Figure 2. It was found that crypts can tolerate higher doses when treated intermittently than constantly.

The magnitude of the advantage has been estimated to be as much as ten years. This estimate depends upon the conversion of computer ticks to human time. We previously estimated that one tick corresponds to approximately 4.5 hours. This was determined by comparing the time that the model crypts recover from a mild perturbation [11], and the time that colon function recovers after chemotherapy [17].

It is less obvious how to prevent spontaneously occurring cancers than how to prevent environmental cancers or hereditary predisposition cancers. The frequency of environmental cancers can be reduced by avoidance, and hereditary cancers can be treated with targeted molecular therapy. But the spontaneously
occurring cancers occur in tissues with more lifetime cell divisions. These spontaneously occurring cancers were previously described as not being appropriate for primary prevention [18], that is the development of precancerous lesions [19]. However, we suggest that the development of colon cancers associated with spontaneous mutations might be delayed by preventing the formation of a precancerous lesion. This could be achieved by reducing the probability that a spontaneous mutant cell would continue to proliferate and become established as an adenoma, an early stage of cancer. The intermittent pulse treatment schedules of an agent that affects the crypt by altering the probability that cells die and are removed at the top of the crypt would achieve primary chemoprevention by delaying the time at which an adenoma would form.

We have modeled the effect of an agent that increases cell death near the top of a crypt by increasing the value of the parameter CptDieMax. An example of such an agent is sulindac that has been reported to increase apoptosis at cells toward the lumen surface [15], and has been reported to be effective in the primary chemoprevention of familial adenomatous polyposis [20]. However, there is toxicity from such anti-inflammatory drugs [21,22], and there has been a failure in the clinical trials of many candidate chemoprevention agents. When used constantly at low doses they may be only modestly effective, and at high doses they may be toxic [20,23]. Intermittent pulse schedules, also termed “short-term intermittent therapy to eliminate premalignancy” (SITEP) have been suggested to overcome the toxic effects of higher doses of candidate chemoprevention agents [4]. The modeling described here suggests how to determine the highest dose in an intermittent pulse
that would be effective in chemoprevention while minimizing the toxic effects of constant high doses.

Patients who might benefit from intermittent pulse treatment schedule would include those with an increased probability of developing colon cancer, including those with an increased probability of developing multiple polyps, aberrant crypt foci [24] or beta-catenin-accumulated crypts (25), and familial adenomatous polyposis [20]. This treatment schedule might also be effective for other tissues that are organized in linear structures with layers of stem cell, proliferating cells, and differentiated cells removed at the top, such as skin and oral mucosa. It is possible that intermittent pulse schedules would be advantageous for preventive treatment of actinic keratosis and for oral leukoplakia, for example.

Conclusions

The relative effectiveness, for the chemoprevention of colon cancer, of intermittent drug schedules compared to constant drug schedules, was quantified by computer simulations of cell dynamics in human colon crypts. Intermittent drug schedules had three times the maximum tolerated dose than constant drug schedules, and could delay the time of the formation of adenomas by 10 years, resulting in chemoprevention by delay.

Ethics approval and consent to participate

The Institutional Review Board of Rutgers University FWA00003913 has approved Protocol #E99-187 relevant to this project. The Institutional Review Board of the
Robert Wood Johnson Medical School of the University of Medicine and Dentistry of New Jersey has approved Protocol 4611, which includes the requirement for informed consent of participants.

**Availability of data and materials**

The datasets supporting the conclusion of this article are included within the article. The computer program with the graphical user interface (Interface tab), program code (Procedures tab) and extensive comments about the model and code (Information tab) is available at http://dx.doi.org/doi:10.7282/T3TQG36W.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ Contributions**

R.B. wrote the core code for the colon crypt computer model and made suggestions for its revision. D.E.A. conceived of the project, revised the code, carried out the simulations, analyzed and interpreted the simulation results, and wrote the manuscript. Both authors approved the manuscript.

**Acknowledgements**

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D.E.A.: ORCID, 0000-0002-0912-3870, axelrod@biology.rutgers.edu

References


aflatoxin-induced tumorigensis and induction of glutathione S-transferase

*Cancer Res.* **55** 4319-24


The selective estrogen receptor modulator arzoxifene and rexinoid LG100268 cooperate to promote transforming growth factor β-dependent apoptosis in breast cancer *Cancer Res.* **64** 3566-71


[18] Tomasetti C, Vogelstein B 2015 Variation in cancer risk among tissues can be explained by the number of stem cell divisions *Science* **347** 78-81


Aberrant crypt foci in colorectal carcinogenesis. Cell and crypt dynamics *Cell Prolif.* **33** 1-18


Table 1. Effect of treatment schedules on time to form an adenoma.

<table>
<thead>
<tr>
<th>Treatment Schedule</th>
<th>N</th>
<th>Ticks (median)</th>
<th>Years (median)</th>
<th>P (within groups)</th>
<th>P (between groups)</th>
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<td>None 1</td>
<td>50</td>
<td>626</td>
<td>0.352</td>
<td>0.97</td>
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<tr>
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<td>624</td>
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<tr>
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<td>10.22</td>
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Years were calculated as 1 tick = 4.5 hours. Nonparametric comparisons for each pair using the Wilcoxon test since the distribution of times in each group was not normally distributed as determined by the Shapiro-Wilk W test.
Table 2. Advantage of intermittent pulse treatment schedule compared to constant treatment for various parameter values.

<table>
<thead>
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<th>Treatment Schedule</th>
<th>Parameter</th>
<th>Time to Adenoma (Median Ticks)</th>
<th>P</th>
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<tr>
<td></td>
<td>Mutant Divide Difference</td>
<td>Proportion</td>
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<tr>
<td>Intermittent</td>
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Other values of Mutant Divide Difference

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Other values of Proportion

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<th>P</th>
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Figure Legends:

Figure 1. Treatment schedules and total cells per crypt as a function of time. Top row, Treatment schedules (Cptdiemax). Bottom row, Total cells per crypt. Left column, no treatment. Middle column, constant treatment. Right column, intermittent pulse treatment. The intermittent treatment (top right) with a pulse of Cptdiemax = 1.4, duration = 3 ticks, and interval = 24 ticks, results in the number of cells per crypt (bottom right) decreasing at each pulse and then returning during the interval between pulses.

Figure 2. Maximum tolerated treatments. Constant schedule, dashed line. Intermittent pulse schedule, solid line. For a constant schedule the maximum tolerated treatment is Cptdiemax = 0.5. For intermittent pulse schedule the maximum tolerated treatment is greater, Cptdiemax = 1.4. For each value of Cptdiemax, 50 independent simulations were run for 2000 ticks. Each point represents one simulation run.

Figure 3. Effect of treatment schedules on numbers of mutant cells per crypt (left) and total number of cells per crypt (right) as a function of time. Top row, no treatment. Middle row, constant treatment. Bottom row, intermittent pulse treatment. Example of one run for each treatment schedule. Mutations are induced at a proportion of $10^{-4}$ per cell per tick. Most mutants and their progeny are flushed
out of the crypt, so their number increases and decreases over time. Stochastically, a rare mutant will establish a stable population of progeny and continue to increase in number. At that time the total number of cells per crypt increases beyond the quasi-stationary number, and the crypt will be filled with mutant cells forming an adenoma. The intermittent pulse treatment schedule greatly delays the time that an adenomatous crypt is formed.

**Figure 4.** Effect of treatment schedules on time to form an adenoma. The box plot indicates the median and 25 and 75 percentiles of each of 50 runs. The whiskers indicate the range of outliers. Notice the log scale. Const = constant treatment; Intermit = intermittent treatment. Intermittent pulse treatment greatly increases the times that adenomas will form. Within group comparisons, $P > 0.2$, between group comparisons, $P < 0.0001$, Wilcoxon nonparametric test. Numerical values are given in Table I.
Figure 1
Figure 2
Figure 4

Time to Adenoma (ticks)

Treatment Schedule

None1, None2, Const1, Const2, Intermit1, Intermit2