Effective Chemotherapy of Heterogeneous and Drug Resistant Early Colon Cancers by Intermittent Dose Schedules: A Computer Simulation Study

(File name: Axelrod CCP Accepted March 1, 2017.pdf)

Cancer Chemotherapy and Pharmacology (2017) 79:889-898
DOI: 10:1007/s00280-017-3272-2
http://rdcu.be/qn25

ORIGINAL ARTICLE

Effective Chemotherapy of Heterogeneous and Drug Resistant Early Colon Cancers by Intermittent Dose Schedules: A Computer Simulation Study

David E. Axelrod[1*], Sudeepti Vedula[2,3], and James Obaniyi[2]

[1]Department of Genetics and Cancer Institute of New Jersey, Rutgers University, 604 Allison Road, Piscataway, NJ 08854-8082, USA.

[2]Department of Biomedical Engineering, Rutgers University, 599 Taylor Road, Piscataway, NJ 08854, USA

[3]Department of Molecular Biology and Biochemistry, Rutgers University, 604 Allison Road, Piscataway, NJ 08854-8082, USA

*Corresponding author: Email: axelrod@biology.rutgers.edu; Telephone: 1-848-445-2011; Fax: 1-732-445-5870

Abstract

Purpose The effectiveness of cancer chemotherapy is limited by intra-tumor heterogeneity, the emergence of spontaneous and induced drug resistant mutant subclones, and the maximum dose to which normal tissues can be exposed without adverse side effects. The goal of this project was to determine if intermittent schedules of the maximum dose that allows colon crypt maintenance could overcome these limitations, specifically by eliminating mixtures of drug resistant mutants from heterogeneous early colon adenomas while maintaining colon crypt function.

Methods A computer model of cell dynamics in human colon crypts was calibrated with measurements of human biopsy specimens. The model allowed simulation of continuous and intermittent dose schedules of a cytotoxic chemotherapeutic drug, as well as the drug's effect on the elimination of mutant cells and the maintenance of crypt function.

Results Colon crypts can tolerate a 10 fold greater intermittent dose than constant dose. This allows elimination of a mixture of relatively drug sensitive and drug resistant mutant subclones from heterogeneous colon crypts. Mutants can be eliminated whether they arise spontaneously or are induced by the cytotoxic drug.

Conclusions An intermittent dose, at the maximum that allows colon crypt maintenance, can be effective in eliminating a heterogeneous mixture of mutant subclones before they fill the crypt and form an adenoma.

Key words Drug resistance, Heterogeneity, Dose schedules, Intermittent, Colon cancer, Adenoma, Crypt

Funding The Human Genetics Institute of New Jersey, the New Jersey Breast Cancer Research Fund, and the Rutgers Cancer Institute of New Jersey (PA30CA072720).

Introduction

Colon cancer starts with abnormal proliferation of cells in the crypt [1]. In normal crypts the rate of proliferation of cells near the bottom of the crypt is balanced by the removal of cells toward the top of the crypt. Cells born at bottom third of the crypt, divide, move up, differentiate in the top two-thirds of the crypt, and are removed at the top [2]. Mutant cells, if they appear, may also move up and out. However, if mutant cells have a higher rate of division than normal cells they may produce a mutant subclone of cells that proliferate so rapidly that the progeny fill the crypt before they are flushed out. This results in an early adenoma, a crypt filled with mutant cells [3]. Treating an early adenoma and eliminating mutant cells before they form an adenomatous crypt may be more effective that treating mutant cells at a later invasive stage of colon cancer.

Mutant cells dividing more rapidly than normal cells are expected to be more sensitive than normal cells to cytotoxic chemotherapeutic drugs such as those currently approved by the FDA for the treatment of colorectal cancer. These include Capecitabine (Xeloda), Fluorouracil (5-FU, Adrucil), Irinotecan (Camptosar), and Oxaliplatin (Eloxatin). Cytotoxic drugs, in addition to killing spontaneously occurring mutant cells, may also induce new mutant cells [4]. Mixtures of mutant cells, whether they are drug-induced or evolve spontaneously, may have a subclones with a range of rates of cell division and a range of drug sensitivities.

Among the challenges of effective chemotherapy is the elimination all types of mutant cells, including relatively drug sensitive cells, relatively drug resistant mutant cells, and heterogeneous mixtures of relatively sensitive and resistant mutant cells. Elimination of mutant cells must be accomplished while reducing the adverse effects of the cytotoxic drugs on the normal cell dynamics that maintains

functional crypts. However, the dose necessary to eliminate all types of mutant cells may be above the maximum dose for which the crypt can maintain the normal number of cells. And lower doses may result in relapse.

Relapse from apparently effective chemotherapy may occur for several reasons. Rapidly dividing mutant cells that are drug sensitive may be eliminated by a given cytotoxic dose, but relapse may occur if relatively slower growing mutant cells resist the cytotoxic dose and emerge at a later time. The slower growing resistant cells may preexist in a heterogeneous population of cells [5] or may be induced by the chemotherapeutic drug [4]. Initial application of a higher dose in anticipation of the emergence of slower growing drug resistant cells may require a dose that exceeds the maximum dose for which the crypt can maintain the normal number of cells.

Several strategies have been employed to reduce the adverse effects of continuous administration of high doses of cytotoxic chemotherapeutic drugs, including modified dose schedules. For instance, metronomic therapy utilizes low doses over long time without breaks [6], adaptive therapy utilizes doses adjusted to the response of tumor to previous doses [7,8]; and intermittent therapy alternates high doses with intervals of low doses, or no therapy, in order to allow recovery [9]. The latter schedules are sometimes called "holidays", "rest periods", "interrupted ", or "stop and go".

Intermittent dose schedules can allow crypts to survive brief periods of higher doses than if the doses were continuous. In between the high doses the relatively resistant quiescent stem cells can emerge from quiescence and repopulate the crypt. However, there is a danger that intermittent dose schedules can also allow resistant mutant cells to recover in between the high doses, resulting in relapse [10]. The use of computer simulation to investigate the possibility of relapse [11] or cure [12,13] under some conditions has been reviewed.

We have tested the hypothesis that intermittent schedules can be effective in eliminating mutant cells from early colon adenomas. In order to test this hypothesis we developed a calibrated computer model of cell dynamics in human colon crypts. The results of computer simulations indicate that intermittent high dose schedules can be effective in eliminating both relatively drug resistant mutant cells and drug sensitive mutant cells, even if the two types of mutants co-exist in heterogeneous colon crypts. Furthermore, resistant and sensitive mutant cells may be eliminated whether they arise spontaneously or are induced by a chemotherapeutic drug.

Methods

Calibrated computer model of human colon crypts

We previously described an agent-based model of normal human colon crypts [14]. The model was calibrated with the number of quiescent stem cells, proliferating cells and non-proliferating differentiated cells measured in human biopsy specimens. Details of image acquisition, measurements by image analysis, and determination of reliability of measurements were previously described in detail [14].

The model assumed that the probability of a cell's proliferating along the crypt axis was determined by its position in a divide gradient in the microenvironment along the crypt axis, with the probability higher toward the bottom than at the top, except for stem cells at the very bottom that are in a quiescent niche. The probability of a cell dying was determined by its position in a die gradient, higher at the top than at the bottom of the crypt. In the presence of a cytotoxic drug a cell has an increased probability of dying determined also by its position in the divide gradient. A mutant cell has an even higher probability of dying than a normal cell at the same position because it has an increased probability of dividing.

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. 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 [15], monoclonal conversion by neutral drift [2], and robust recovery from perturbation by exposure to a cytotoxic agent [16]. Based upon the kinetics of recovery of the model and of humans [17] one computer "tick" was estimated to correspond to approximately 4.5 hours human time.

We previously described a model of an adenomatous crypt with a homogenous population of a single type of mutant [14]. A new enhanced version of the model used here adds the capability to simulate a heterogeneous adenomatous crypt containing multiple mutant subclones with different probabilities of dividing and different probabilities of dying. The new model "Colon Crypt Model 110514 G.nlogo" is available as an open source program, see Supplementary files. Details of the virtual crypt model, including graphical user interface (Interface tab), computer code (Procedures tab), and extensive comments (Information tab), are included.

The virtual crypt model was produced with the NetLogo application v.4.1.3, and was run on v.4.1.3 and v.5.3.1. 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/.

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, JMP Pro 11, or JMP Pro 12 (SAS Institute, Cary, NC 27513, USA) to generate plots and for statistical analysis. Simulations and analysis were done on an iMac computer or MacBookPro computer running OS 10.9.5, or OS 10.11.5.

Results

Intermittent Chemotherapy Dose Schedules

The response of colon crypts to constant or intermittent dose schedules of a cytotoxic chemotherapeutic drug over time can be simulated with the virtual colon crypt model. Human colon crypts have an average of 2428 cells, as measured in human biopsy specimens. In simulations, the number of cells per crypt varies slightly over time in the absence of chemotherapy, but in the presence of constant chemotherapy the number of cells per crypt rapidly decreases to zero and the crypt collapses (Fig. 1, left). However, if the chemotherapy is intermittent the number of cells per crypt does not decrease to zero, but rather oscillates around the average value and the crypt is maintained (Fig. 1, right.).

In this example the intermittent dose schedule had a duration of 6 ticks, an interval between the start of one dose and the next dose equal to 32 ticks, and the lethality of the dose equal to 2. One tick is equivalent to 4.5 hours human time. The values of interval and duration are similar to those previously determined to minimize collateral damage to normal cells and to minimize the time to eliminate mutant cells [14]. The probability that a cell will die in the absence of a drug is determined by its position along the crypt axis. Lethality is a parameter that increases the probability that a cell will die in the presence of a cytotoxic drug. Mutant cells have an increased probability of dividing compared to normal cells located at the same position along the crypt axis and are more sensitive to the lethal effects of the cytotoxic chemotherapeutic drug.

The lethal dose that a crypt can survive is different for constant dose schedules and intermittent schedules. The difference is shown in Fig. 2. For constant dose schedules greater than lethality = 0.2 the number of cells per crypt is reduced to zero and the crypt collapses, whereas for intermittent schedules the crypts are stable to at least lethality = 2.0. The maximum dose at which a crypt is maintained is about 10 times more for intermittent schedules than constant schedules.

Normal cells, most of which are born in the region of proliferating cells near the bottom third of the crypt, move up and produce progeny that are removed at the top. Mutant cells will also move toward the top of the crypt. However, a mutant that has 1.16 times the probability of dividing than a normal cell at the same position in the crypt, will produce progeny that move toward the top of the crypt but may not be flushed out at the top of the crypt. Rather, the progeny may increase in number and fill the crypt with mutant cells forming an early adenoma (Fig. 3, left). Constant chemotherapy at the maximum dose that will maintain the number of normal cells in the crypt will delay, but not eliminate, the mutant cells from filling the crypt (Fig. 3, middle). However, intermittent chemotherapy at the higher dose that can maintain the average number of normal cells will reduce the number of mutant cells to zero (Fig. 3, right).

Therefore, intermittent chemotherapy at the maximum dose that allows maintenance of the normal number of cells in the crypt can be effective in eliminating rapidly growing mutant cells.

Intermittent dose schedules can eliminate both fast growing drug sensitive and slow growing drug resistant cells in a heterogeneous crypt

A crypt may be heterogeneous, containing different cells with different genetic mutations [18] and different drug responses. For example, Mutant A which grows 1.16 times faster than normal cells, and Mutant B which grows 1.08 times faster than normal cells. The survival of these two mutants exposed to different lethal treatments of chemotherapy is shown in Fig. 1 Supplement. The faster growing Mutant A is more sensitive to the chemotherapeutic drug than Mutant B.

The relative growth rate of the two mutants coexisting in a heterogeneous crypt is shown in Fig. 4, left. When the crypt is exposed to the maximum intermittent dose, the number of each mutant is reduced to zero (Fig. 4, right). Mutants with growth rates other than the two in this example are also reduced to zero by the intermittent dose schedule, Fig. 2 Supplement.

Therefore, intermittent chemotherapy at the maximum dose that allows maintenance of the normal number of cells in the crypt can be effective in eliminating from a heterogeneous crypt both slow growing relatively drug resistant mutant cells and faster growing relatively drug sensitive mutant cells.

Intermittent doses schedules can eliminate spontaneously evolving mutants in a heterogeneous crypt

Many different mutants may arise early in tumor progression [19]. Early mutants may be unstable [20] and capable of evolving other mutants that may have different drug resistance [21]. For instance, rapidly growing mutant cells may spontaneously evolve mutant subclones with slower growth rates that are more drug resistant (Fig. 5, top, left). A crypt with a rapidly growing mutant cell may be detected as an abnormal crypt and treated with intermittent chemotherapy in order to eliminate the rapidly growing mutant cell. The slow growing mutant cell might not be detected when the rapidly growing cells are eliminated. Nevertheless, an intermittent dose schedule that eliminates the rapidly growing relatively drug sensitive mutant cells, can also eliminate the slow growing relatively resistant "sleeper" mutant cells, if the intermittent chemotherapy is continued beyond the time that the rapidly growing mutant cells are eliminated (Fig. 5, top, right.)

In a complementary situation, slow growing mutant cells may spontaneously evolve more rapidly growing cells. When the rapidly growing cells are detected it may not be apparent that the crypt is heterogeneous and that it has also contained slow growing relatively resistant mutant cells (Fig. 5, bottom, left). Nevertheless, an intermittent dose schedule of a chemotherapy drug intended to eliminate the rapidly dividing drug sensitive cells can also eliminate the undetected slow growing drug resistant cells (Fig. 5, bottom, right).

Therefore, intermittent chemotherapy at the maximum dose that allows maintenance of the normal number of cells in the crypt can be effective in eliminating both parental mutants and mutants that spontaneously evolve from them.

Intermittent dose schedules can eliminate drug-induced mutants in a heterogeneous crypt

Cytotoxic chemotherapeutic drugs may be mutagenic [4]. A drug used to treat a crypt with the intention of eliminating rapidly growing mutant cells may also induce new subclones of slow growing relatively resistant mutants. Nevertheless, chemotherapeutic drugs, in spite of their mutagenicity, when given with intermittent dose schedules, can eliminate both sensitive parental cells and drug-induced resistant mutant cells (Fig. 6, left).

Similarly, a mutagenic chemotherapeutic drug given with the intention of eliminating slow growing mutant cells in a crypt may also induce a more rapidly growing subclone. Nevertheless, the drug given intermittently can eliminate both the parental slow growing mutant and the drug-induced rapidly growing subclone (Fig. 6, right.)

Therefore, intermittent dose schedules of a mutagenic chemotherapeutic drug that induces new mutants as well as killing mutant cells, can be effective in eliminating the drug-induced mutants and the parental mutants.

Discussion

Results of computer simulation indicate that an intermittent schedule of the maximum dose of a cytotoxic drug that maintains the normal number of cells in a crypt can be effective in eliminating a mixture of mutant cells from a heterogeneous colon crypt. This can be accomplished whether the mutant cells preexist in the crypt or are induced by the cytotoxic drug.

During the maximum intermittent dose normal dividing cells and mutant dividing cells are killed by the cytotoxic drug, but the quiescent stem cells are relatively resistant. In between doses, the reservoir of normal quiescent stem cells responds to the reduction in the number of normal cells and emerges to proliferate and restore the usual number of cells in the crypt. Mutant cells that are killed do not have a reservoir from which to recover, so could be eliminated. A mixture of mutant cells, with different rates of division in a heterogeneous crypt, could also eliminated.

A colon crypt with abnormal dividing mutant cells is an early step the sequence from adenoma [3] to invasive colon cancer [1]. These early mutations precede [22,23] the large number of mutations found in clinical tumors [18]. The simulation results reported here indicate that targeting mutant cells in early adenomas with maximum intermittent doses of cytotoxic chemotherapeutic drugs could eliminate a mixed population of mutants before they proliferate to spawn further mutant cells that result in invasive carcinoma.

Mutant cells may spontaneously become resistant to cytotoxic drugs by a variety of molecular mechanisms [24]. Also, new mutants may be induced by cytotoxic drugs. Mathematical models have been used to described the generation and elimination of drug resistant mutants [25,26]. Results of computer simulations indicate that both relatively rapid growing drug sensitive mutants, and relatively slow growing drug resistant mutants, can both be eliminated by intermittent drug doses at the maximum that will maintain the number of cells in the crypt. Such intermittent doses can eliminate mutant cells that pre-exist before drug treatment, and eliminate new mutants that are induced by the drug treatment.

Intermittent dose schedules where introduced for radiotherapy as early as 1911 [27], and have been further refined and commonly implemented as fractionation schedules [28]. Intermittent chemotherapy was employed in the early 1970's for the MOMP and MOPP protocols for Hodgkin's disease [29], adjusting timing of treatment schedules to allow bone marrow recovery after cytotoxic chemotherapy.

Intermittent treatment schedules have also been applied for chemotherapy of other cancers, for instance breast [30], renal cell carcinoma [31], and prostate [32].

There have been several clinical trials comparing intermittent and continuous chemotherapy of advanced and metastatic colon cancer. Maughan et al. [33] reported for a multicenter randomized trial of advance colorectal cancer that, for two day intervals of fluoruracil, there was no clear evidence of difference of overall survival, but significantly fewer toxic effects and serious adverse events. Van Cutsem et al. [34] used an oral fluoropyrimidine carbamate (Capecitabine) two weeks on and one week off in a randomized phase II study of advanced colorectal cancer; based on observations of toxicity, dose-intensity, response rate, and time to disease progression, they suggested a phase III trial with intermittent dose schedules. Tournigand et al. [35] investigated the use of oxaliplatin discontinuation and reintroduction in a stop-and-go strategy in order to reduce severe neurotoxicity in advance colorectal cancer; they concluded that oxaliplatin could be safely stopped after six cycles of a combination of leucovorin and fluoruracil. Adams et al. [36] evaluated intermittent vs. continuous oxaloplatin and fluoropyrimidine combination against advanced colorectal cancer focusing on the length and quality of life with the intention by reducing toxicity; they found that for patients with normal baseline platelet counts that there were benefits to intermittent treatment without reducing survival. In a recent review and meta-analysis of continuous vs. intermittent chemotherapy for metastatic colorectal colon cancer Berry et al. [37] concluded that there was no statistically significant difference in overall survival and therefore intermittent chemotherapy dose schedules should be considered as a treatment option.

Our intermittent chemotherapy studies also are concerned with colon cancer, but differ from the chemotherapy clinical trials discussed above. First, these clinical trials were concerned with the survival of patients with advanced and metastatic colorectal cancer, whereas our study is focused on pre-malignant adenomas and the intervention in the early stages in the evolution of colon cancer. Second, the intermittent time periods that were considered in these trials are weeks to months, whereas the time periods that we have suggested are of the order 1 day dose and 6 day interval. Third, we have considered the proliferation of normal cells and mutant cells in an intact crypt of about 2400 cells, whereas the clinical trials are concerned with a tumor mass of more than a million cells. Our results support the suggestion of Blackburn [38], that early “interception” of tumor evolution by eliminating a few mutant cells in a crypt can be more effective than late therapy that tries to eliminate millions of mutant cells in a tumor.

Intercepting the early stages in the evolution of pre-malignant adenomas to invasive cancer would require the detection of early adenomas [39]. Patients with familial adenomatous polyposis are at high risk [40]. Early adenomas could be identified as aberrant crypt foci [41], crypts that accumulate beta-catenin [42], crypts with altered mucin expression [43], or crypts with abnormalities detected by magnetic resonance molecular imaging [44].

In summary, using a computer model we have obtained simulation results that indicate that intermittent doses of a cytotoxic chemotherapeutic drug, at the maximum dose that maintains the number of normal cells in a crypt, could eliminate mutant cells while sparing normal crypt function, and thereby prevent early adenomatous crypts from evolving into invasive colon cancer. Intermittent dose schedules could be effective in eliminating mixtures of mutant cells from heterogeneous crypts, whether the mutants arise spontaneously before drug treatment, or are drug-induced.

Authors' Contributions D.E.A. conceived of the project, analyzed and interpreted the simulation results, and wrote the manuscript. S.V. and D.E.A. carried out the simulations. J.O. and D.E.A. wrote the revised the code. All authors approved the manuscript.

Acknowledgements We thank Rafael Bravo for writing the code for the original colon crypt model, Dr. Steven Schiff for providing human biopsy specimens, members of the Division of Life Sciences IT Support Group for computer services, RUCore staff for file archive services, and Uri Wilensky for making available the NetLogo open-source application.

Funding The Human Genetics Institute of New Jersey, the New Jersey Breast Cancer Research Fund, and the Rutgers Cancer Institute of New Jersey (PA30CA072720).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval 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.

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

Supplementary files:

Fig 1 Supplement, Survival of Mutant A and Mutant B.

Fig 2 Supplement, Time to cure for mutants of different growth rates.

Colon Crypt Model 110514 G. nlogo. The model program is available to download at http://dx.doi.org/doi:10.7282/T3KH0QKV. The model program runs on the open-source multi-platform NetLogo application, version 4.1.3 or 5.1.3, available to download at http://ccl.northwestern.edu/netlogo/.

References

1. Leslie A, Carey FA, Pratt NR, Steele RJC (2002) The colorectal adenoma-carcinoma sequence. Br J Surg 89:845-860

2. Humphries A, Wright NA (2008) Colonic crypt organization and tumorigenesis. Nat Rev Cancer 8:415-425. doi: http://dx.doi.org/10.1038/nrc2392

3. Strum WB (2016) Colorectal adenomas. N Engl J Med 371:1065-1075. doi: http://dx.doi.org/10.1056/NEJMc1604867

4. Benedict WF, Baker MS, Haroun L, Choi E, Ames BN (1977) Mutagenicity of cancer chemotherapeutic agents in the Salmonella/microsome test. Cancer Res 37:2209-2213

5. Marusyk A, Polyak K (2010) Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 1805:105-117 . doi: http://dx.doi.org/10.1016/j.bbcan.2009.11.002

6. Pasquier E, Kavallaris M, Andre N (2010) Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 7:455-465. doi: http://dx.doi.org/10.1038/nrclinonc.2010.82

7. Gatenby RA, Silva AS, Gillies RJ, Frieden BR (2009) Adaptive therapy. Cancer Res 69:4894-4903. doi: http://dx.doi.org/10.1158/0008-5472.CAN-08-3658

8. Enriquez-Navas PM, Kam Y, Das T, Hassen S, Silva A, Foroutan P, Rulz E, Martinez G, Minton S, Gilles RJ, Gatenby RA. (2016). Exploiting evolutionary principles to prolong tumor control in preclinical models of breast cancer. Sci Trans Med 8:325ra24. doi: www.sciencetranslationalmedicine.org/cgi/content/full/8/327/327ra24/DC1

9. Leder K, Pitter K, Laplant Q, Hambardzumyan D, Ross BD, Chan TA, Holland EC, Michor F (2014) Mathematical modeling of PDGF-driven glioblastoma reveals optimized radiation dosing schedules. Cell 156:603-616. doi: http://dx.doi.org/10.1016/j.cell.2013.12.029

10. Kim JJ, Tannock IF (2005) Repopulation of cancer cells during therapy: an important cause of treatment failure. Nat Rev Cancer 5:516-525

11. Marcu L, Bezak E (2010) Modeling of tumour repopulation after chemotherapy. Australas Phys Eng Sci Med 33:265-270

12. Venkatakrishnan K, Friberg LE, Ouellet D, Mettetal JT, Stein A, Trocóniz IF, Bruno R, Mehrotra N, Gobburu J, Mould DR (2015) Optimizing oncology therapeutics through quantitative translational and clinical pharmacology: challenges and opportunities. Clin Pharmacol Ther 97:37-54. doi: http://dx.doi.org/10.1002/cpt.7

13. Shi J, Alagoz O, Erenay FS, Su Q (2014) A survey of optimization models on cancer chemotherapy treatment planning. Ann Oper Res 221:331-356. doi: 10.1007/s10479-011-0869-4

14. Bravo R, Axelrod DE (2013) A calibrated agent-based computer model of stochastic cell dynamics in normal human colon crypts useful for in silico experiments. Theoretical Biology & Medical Modelling 10:66. doi: http://10.1186/1742-4682-10-66

15. Wright NA (2006) Review article: is there a common principle in the development of gastrointestinal cancers? Stem cells in the origin of cancer. Aliment Pharmacol Ther 24 Suppl 4:31-40

16. Potten CS (1990) A comprehensive study of the radiobiological response of the murine (BDF1) small intestine. Int J Radiat Biol 58:925-973

17. Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, Kambe M, Taguchi T, Ogawa N (1993) Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. CPT-11 Gastrointestinal Cancer Study Group. J Clin Oncol 11:909-913

18. Borras E, San Lucas FA, Chang K, Zhou R, Masand G, Fowler J, Mor ME, You YN, taggart MW, McAllister F, Jones DA, Davies GE, Edelmann EA, Ehli EA, Lynch PM, Hwak ET, Capella G, Scheet PI, Vlar E (2016). Genomic landscape of colorectal mucosa and adenomas. Cancer Prev Res 9:417-427

19. Sottoriva A, Kang H, Ma Z, Graham TA, Salomon MP, Zhao J, Marjoram P, Siegmund K, Press MF, Shibata D, Curtis C (2015) A Big Bang model of human colorectal tumor growth. Nat Genet 47:209-216. doi: http://dx.doi.org/10.1038/ng.3214

20. Charames GS, Bapat B (2003) Genomic instability and cancer. Curr Mol Med 3:589-596

21. Oddo D, Sennott EM, Barault L, Valtorta E, Arena S, Cassingena A et al (2016) Molecular landscape of acquired resistance to targeted therapy combinations in *BRAF*-mutant colorectal cancer. Cancer Res 76:4504-4515

22. Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B (2001) Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 61:818-822

23. Tomasetti C, Vogelstein B, Parmigiani G (2013) Half or more of the somatic mutations in cancers of self-renewing tissues originate prior to tumor initiation. Proc Natl Acad Sci U S A 110:1999-2004. doi: http://dx.doi.org/10.1073/pnas.1221068110

24. Panczyk M (2014) Pharmacogenetics research on chemotherapy resistance in colorectal cancer over the last 20 years. World J Gastroenterol 20:9775-9827. doi:10.1038/nrc3599

25. Foo J, Michor F (2014) Evolution of acquired resistance to anti-cancer therapy. J Theor Biol 355:10-20. doi: http://dx.doi.org/10.1016/j.jtbi.2014.02.025

26. Komarova N. (2006) Stochastic modeling of drug resistance in cancer. J Theor Biol 239:351-366

27. Cox JD (1988) Time, dose, and fractionation in radiation therapy: An historical perspective. In Vaeth JM, Meyer J (eds) Time, Dose and Fractionation in the Radiation Therapy of Cancer: A Frontier Revisited. Karger, Basel, pp 14-18

28. Ahmed KA, Correa CR, Dilling J, Rao NG, Shridhar R. Trotti AM, Wilder RB, Caudell, JJ (2014) Altered fractionation schedules in radiation treatment: A review. Semin Oncol 41:430-750. doi: http://dx.doi.org/10.1053/j.seminoncol.2014.09.012

29. DeVita V, Chu E (2008) A history of cancer chemotherapy. Cancer Res 68:8643-8653. doi: 10.1158/0008-5472.CAN-07-6611

30. Beex L, Rose C, Mouridsen H, Jassem J, Nooij M, Estape J, Paridaens R, Piccart M, Gorlia T, Lardenoije S, Baila L (2006) Continuous versus intermittent tamoxifen versus intermittent/alternated tamoxifen and medroxyprogesterone acetate as first line endocrine treatment in advanced breast cancer: an EORTC phase III study (10863). Eur J Cancer 42:3178-3185

31. Vázquez S, León L, Fernandez O, Lázaro M, Grande E, Aparicio L (2012) Sunitinib: the first to arrive at first-line metastatic renal cell carcinoma. Adv Ther 29:202-217. doi: http://dx.doi.org/10.1007/s12325-011-0099-9

32. Gruca D, Bacher P, Tunn U (2012) Safety and tolerability of intermittent androgen deprivation therapy: a literature review. Int J Urol 19:614-625. doi: http://dx.doi.org/10.1111/j.1442-2042.2012.03001.x

33. Maughan TS, James RD, Kerr DJ, Ledermann JA, Seymour MT, Topham C, McArdle C, Cain D, Stephens RJ, Medical Research Council Colorectal Cancer Group (2003) Comparison of intermittent and continuous palliative chemotherapy for advanced colorectal cancer: a multicentre randomised trial. Lancet 361:457-464

34. Van Cutsem E, Findlay M, Osterwalder B, Kocha W, Dalley D, Pazdur R, Cassidy J, Dirix L, Twelves C, Allman D, Seitz JF, Schölmerich J, Burger HU, Verweij J (2000) Capecitabine, an oral fluoropyrimidine carbamate with substantial activity in advanced colorectal cancer: results of a randomized phase II study. J Clin Oncol 18:1337-1345

35. Tournigand C, Cervantes A, Figer A, Lledo G, Flesch M, Buyse M, Mineur L, Carola E, Etienne P, Rivera F, Chirivella I, Perez-Staub N, Louvet C, André T, Tabah-Fisch I, de Gramont A (2006) OPTIMOX1: a randomized study of FOLFOX4 or FOLFOX7 with oxaliplatin in a stop-and-Go fashion in advanced colorectal cancer--a GERCOR study. J Clin Oncol 24:294-440

36. Adams RA, Meade AM, Seymour MT, Wilson RH, Madi A, Fisher D, Kenny SL, Kay E, Hodgkinson E, Pope M, Rogers P, Wasan H, Falk S, Gollins S, Hickish T, Bessell EM, Propper D, Kennedy MJ, Kaplan R, Maughan TS, MRC COIN Trial Investigators (2011) Intermittent versus continuous oxaliplatin and fluoropyrimidine combination chemotherapy for first-line treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet Oncol 12:642-653. doi: http://dx.doi.org/10.1016/S1470-2045(11)70102-4

37. Berry SR, Cosby R, Asmis T, Chan K, Hammad N, Krzyzanowska MK. (2015) Continuous versus intermittent chemotherapy strategies in metastatic colorectal cancer: a systematic review and meta-analysis. Ann Oncol 26:477-485

38. Blackburn EH (2011) Cancer interception. Cancer Prevention Research 4:787-792. doi: http://10.1158/1940-6207.CAPR-11-0195

39. Corley DA, Levin TR, Doubeni CA (2014) Adenoma detection rate and risk of colorectal cancer and death. N Engl J Med 370:1298-1306. doi: http://dx.doi.org/10.1056/NEJMc1405329

40. Cruz-Correa M, Giardiello FM (2002) Diagnosis and management of hereditary colon cancer. Gastroenterol Clin North Am 31:537-549

41. Roncucci L, Stamp D, Medline A, Cullen JB, Bruce WR (1991) Identification and quantification of aberrant crypt foci and microadenomas in the human colon. Hum Pathol 22:287-294

42. Kuno T, Yamada Y, Hirose Y, Katayama M, Sakata K, Hara A, Saji S, Mori H (2002) Induction of apoptosis by sulindac in azoxymethane-induced possible colonic premalignant lesions in rats. Jpn J Cancer Res 93:242-246

43. Krishn SR, Kaur S, Smith LM, Johansson SL, Jain M, Patel A, Gautam SK, Hollingsworth MA, Mandel U, Clausen H, Lo WC, Fan WT, Manne U, Batra SK (2016). Mucins and associated glycan signatures in colon adenoma-carcinoma sequence: Prospective pathological implications(s) for early diagnosis of colon cancer. Cancer Lett 374:304-341

44. Rossez Y, Burtea C, Laurent S, Gossset P, Léonard R, Gonzalez W, Ballet S, Raynal I, Rousseaux O, Dugué T, vander Elst L, Jichalski J-C, Muller RN Robbe-Masselot C (2016) Early detection of colonic dysplasia by magnetic resonance molecular imaging with a contrast agent raised against colon cancer marker MUC5AC. Contrast Media Mol Imaging 11:211-221

Figure legends

Fig. 1 Chemotherapy dose schedules (top) and number of cells per crypt (bottom) Left: crypts cannot tolerate a constant dose of Lethality = 2. The number of cells per crypt is reduced to zero. Right: crypts can tolerate an intermittent dose schedule with Lethality = 2, Interval = 32, and Duration = 6. The number of cells per crypt oscillates about the normal number of cells per crypt

Fig. 2 Number of cells per crypt as a function of treatment The normal number of cells per crypt can be maintained with an intermittent schedule of up to a maximum dose of Lethality = 2 (—). But with a constant schedule the maximum dose is 10x less, Lethality = 0.2 (-----). The intermittent dose schedule had an Interval = 32 and Duration = 6. Each point is the result of one of 50 runs

Fig. 3 Intermittent dose schedule is more effect than constant dose schedule Mutant A is introduced at 200 ticks. Left: without chemotherapy the number of mutants increases to fill the crypt. Middle: with maximum constant dose initiated when the number of mutant cells = 25% of the total cells, the number of mutant cells also increases to fill the crypt although it takes longer. Right: with maximum intermittent dose, the number of mutant cells decreases to zero, and the crypt is "cured". Each panel is one example of 50 runs with similar results

Fig. 4 Two subclones in a heterogeneous adenoma can both be eliminated by intermittent dose schedules of chemotherapy Rapidly growing Mutant A (———) and slow growing Mutant B (------). Left: without chemotherapy Mutant A fills the crypt. Right: with intermittent chemotherapy initiated when the number of Mutant A cells is 25% of the number of cells of Mutant A and Mutant B are each reduced to zero. Each panel is of one of 50 runs with similar results

Fig. 5 Spontaneously evolving and parental mutant subclones both can be eliminated by intermittent dose schedules. Top, left: in the absence of chemotherapy, while the rapidly growing mutant subclone Mutant A (———) begins to

fill the crypt, the slow growing subclone Mutant B (------) evolves from Mutant A. Top, right: intermittent chemotherapy initiated when the number of Mutant A cells is 25% of the total number of cells in the crypt, results in the number of both subclones reduced to zero. Bottom, left: in the absence of chemotherapy, the slow growing Mutant B increases in number, and at a later time the rapidly growing Mutant A spontaneously evolves from Mutant B and fills the crypt. Bottom, right: intermittent chemotherapy initiated when the number of Mutant A cells is 25% of the total number of cells in the crypt, results in the number of both subclones reduced to zero. Each panel is one of 50 runs with similar results

Fig. 7 Drug-induced mutant subclones and their parent both can be eliminated by intermittent dose schedules. Left: when Mutant A (———) reached 25% of the total number of cells in the crypt, intermittent chemotherapy was started. The chemotherapeutic drug induced Mutant B (------) from Mutant A. Even though Mutant B was slow growing and relatively resistant, both Mutant B and Mutant A were cured by intermittent chemotherapy. Right. When Mutant B (------) reached 25% of the total cells in the crypt, intermittent chemotherapy was started. The chemotherapeutic drug induced Mutant A (——) from Mutant B. Even though Mutant A was more rapidly growing, the number of both Mutant B and Mutant A were reduced to zero by intermittent chemotherapy. This is one example in which both Mutant A and Mutant B were cured in 96% (48/50) of such simulation runs

Fig. 1 Supplement Survival of Mutant A (—) and Mutant B (-------) treated with a single dose, duration = 6, of the indicated lethal treatments Mutant A grows faster than normal cells and has a higher probability of spontaneously of dying. Its Mutant Divide Difference = 0.16 and Mutant Die Difference = 0.1. Mutant B grows slower than Mutant A and has a lower spontaneous probability of dying than Mutant A, its Divide Difference = 0.08 and Mutant Die Difference = 0.05. Lethality is a value that increases the probability that a cell, at a given position in the crypt, will die. At Treatment Lethality = 2, slow growing Mutant B is 4X more resistant to chemotherapy treatment than Mutant A

Fig. 2 Supplement Parameter Sensitivity Analysis The effect of mutant growth rate on the time between the start of chemotherapy and the time at which the number of mutants is reduced to zero. Chemotherapy was started when the percent of mutants in the crypt was 25% (•) or 45% (**O**). Each point is the average of 10 runs. Intermittent chemotherapy was effective in curing mutants that grow at any rate

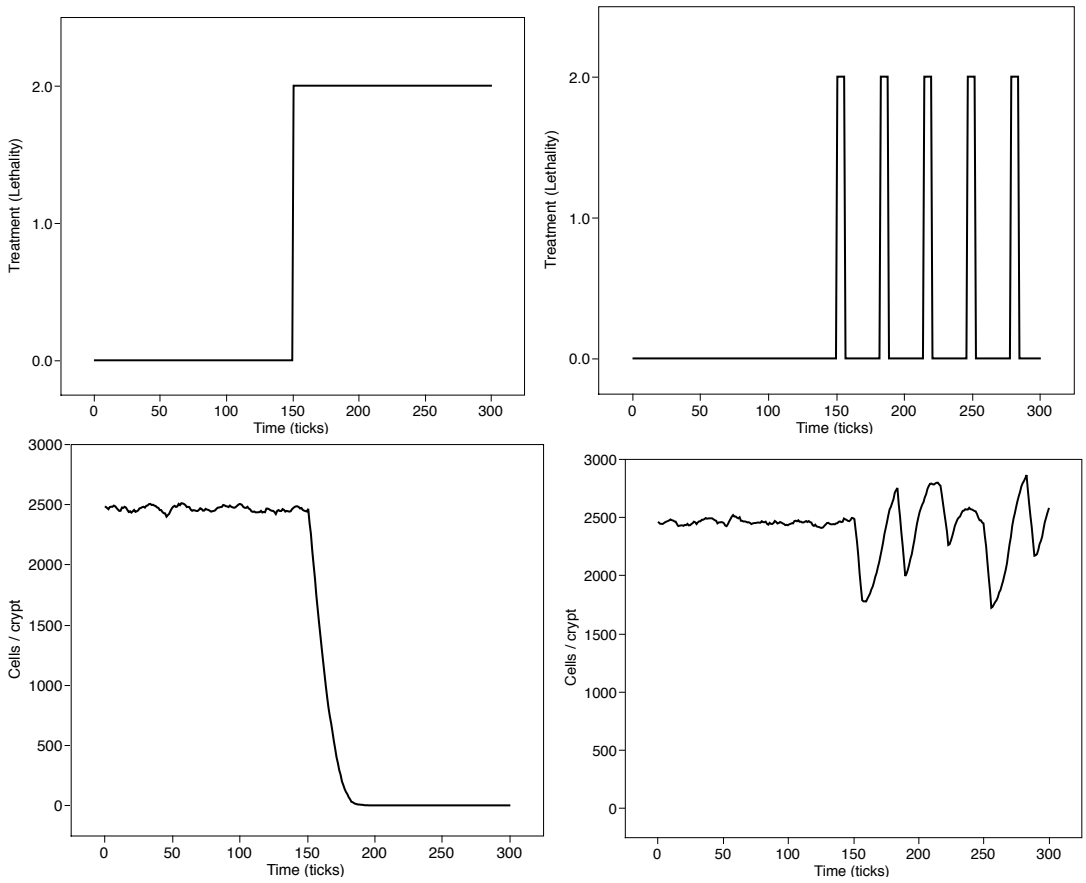
Treatment (Lethality)
2.0
1.0
0.0
0
50
100
150
200
250
300
Time (ticks)
Cells / crypt
3000
2500
2000
1500
1000
500
0

Fig. 1

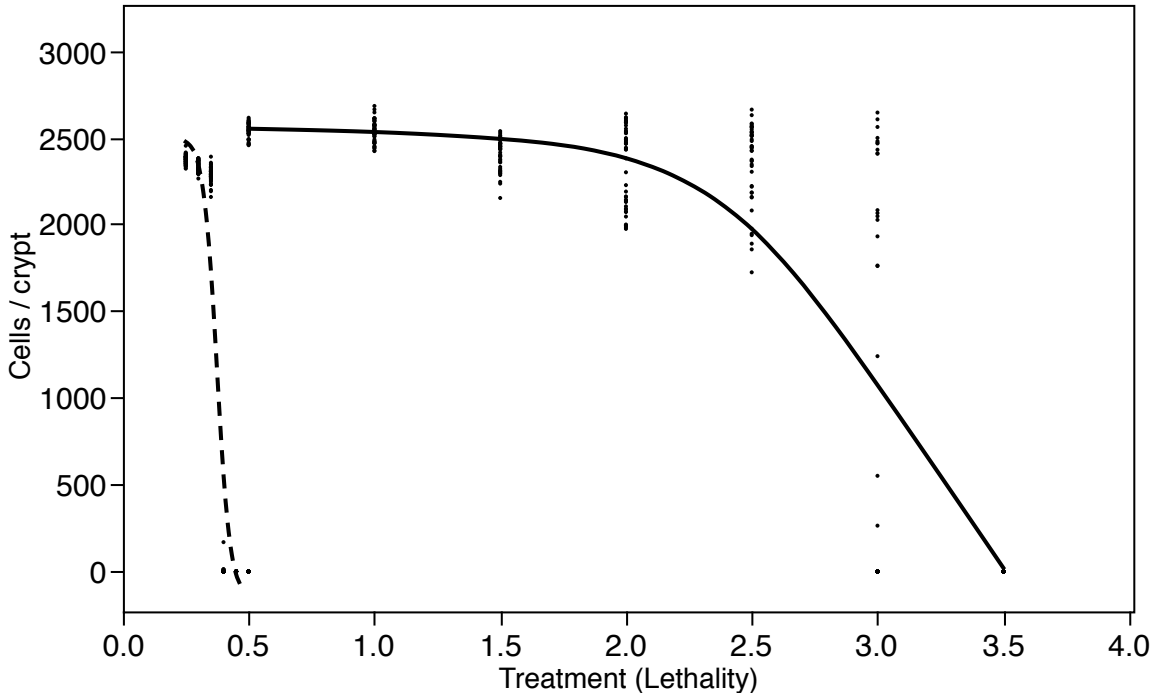
3000
2500
2000
1500
1000
500
0
Cells / crypt
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Treatment (Lethality)

Fig. 2

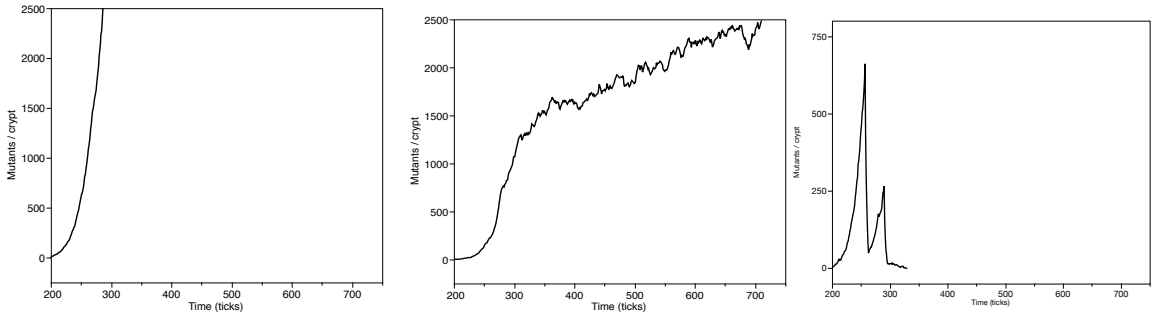
Mutants / crypt
Time (ticks)
2500
2000
1500
1000
500
0
200
300
400
500
600
700
750
250

Fig. 3

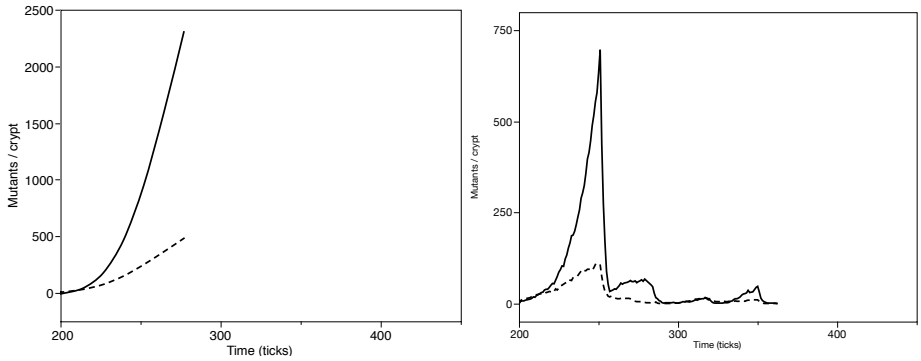
2500
2000
1500
1000
500
0
Mutants / crypt
200
300
400
Time (ticks)
750
500
250
0
Mutants / crypt
200
300
400
Time (ticks)

Fig. 4

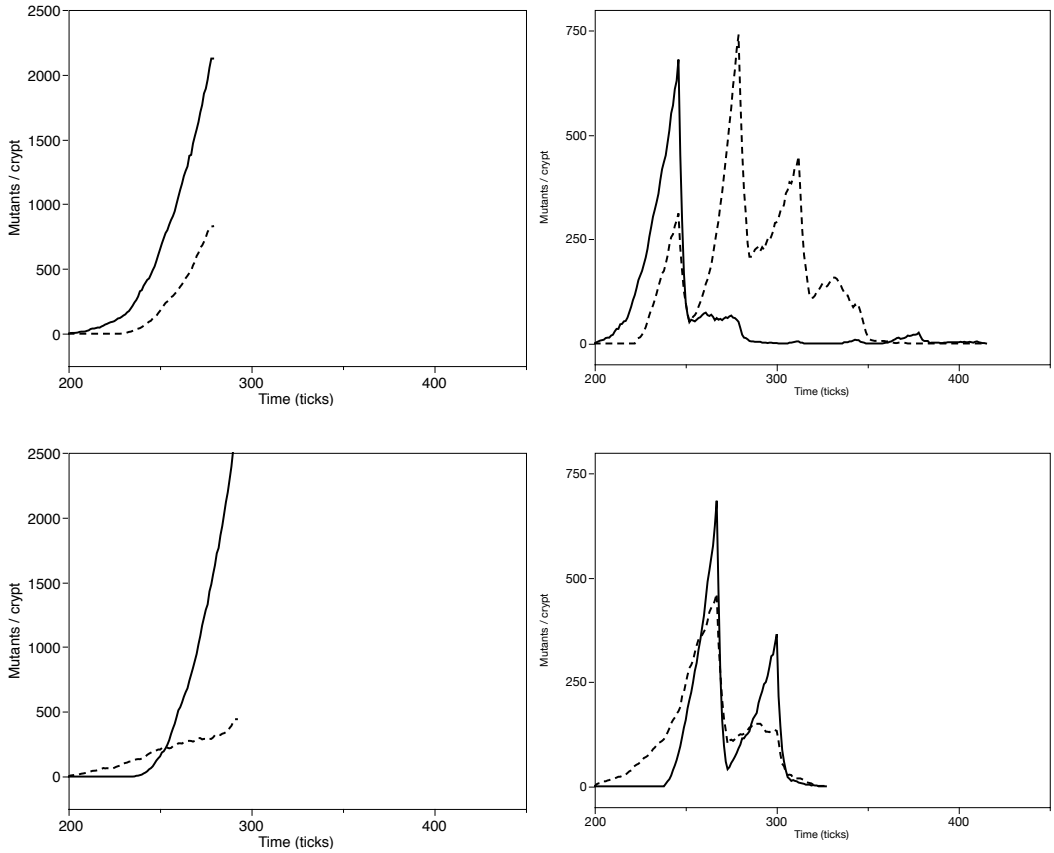
Mutants / crypt
Time (ticks)
2500
2000
1500
1000
500
0
750
250
200
300
400

Fig. 5

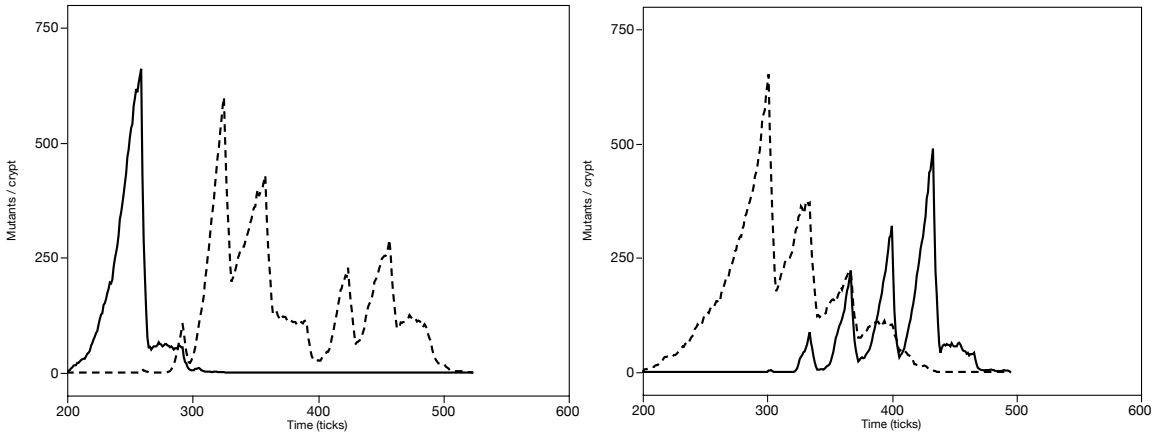
750
500
250
0
Mutants / crypt
200
300
400
500
600
Time (ticks)
750
500
250
0
Mutants / crypt
200
300
400
500
600
Time (ticks)

Fig. 6

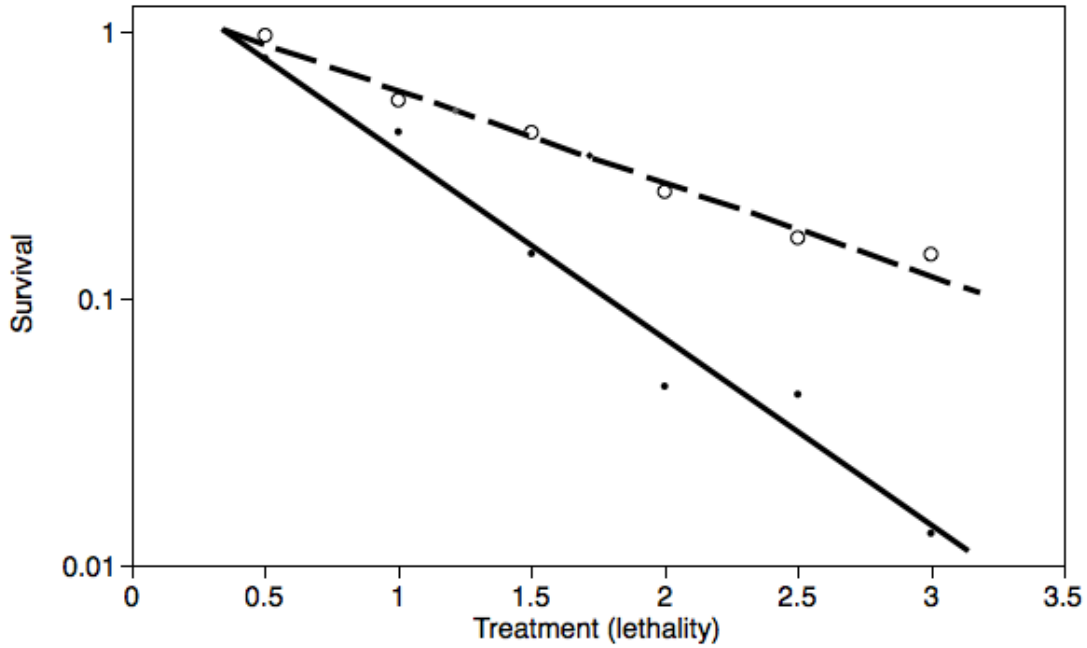

Fig. 1 Supplement

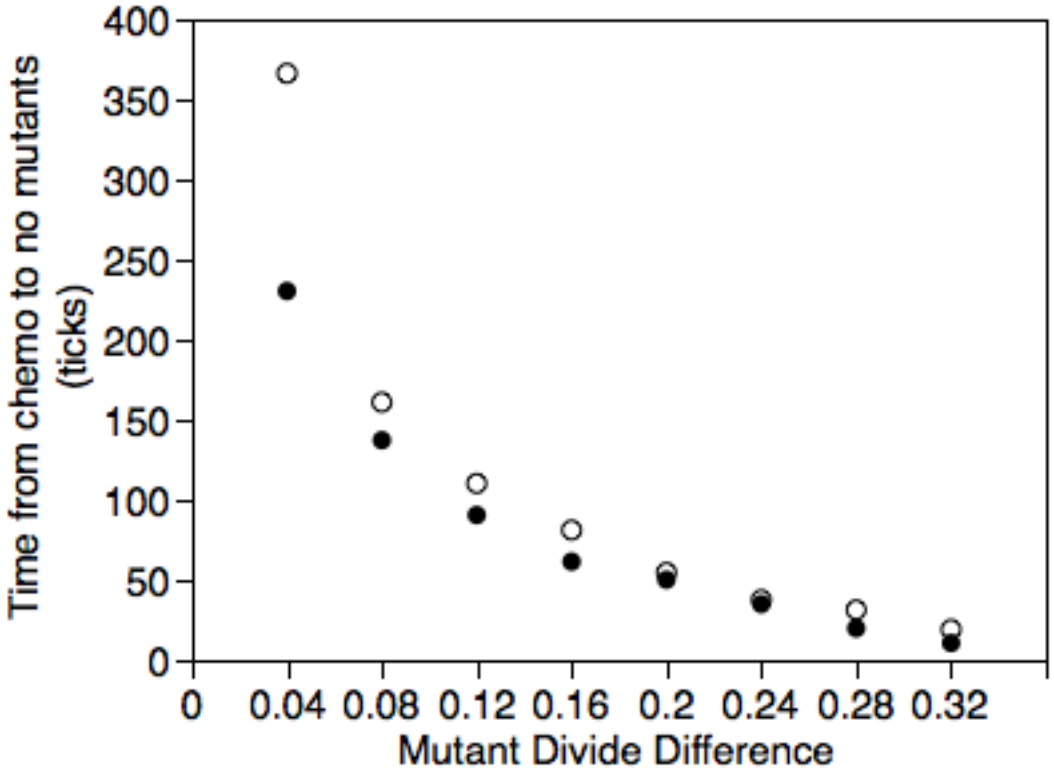

Fig. 2 Supplement