Development of Adaptive Therapy Protocols for Cancer

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2023 by the
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May 2023

ABSTRACT

Resistance to existing anti-cancer drugs poses a key challenge in the field of medical oncology, in that it results in the tumor not responding to treatment using the same medications to which it responded previously, leading to treatment failure. Adaptive therapy utilizes evolutionary principles of competitive suppression, leveraging competition between drug resistant and drug sensitive cells, to keep the population of drug resistant cells under control, thereby extending time to progression (TTP), relative to standard treatment using maximum tolerated dose (MTD). Development of adaptive therapy protocols is challenging, as it involves many parameters, and the number of parameters increase exponentially for each additional drug. Furthermore, the drugs could have a cytotoxic (killing cells directly), or a cytostatic (inhibiting cell division) mechanism of action, which could affect treatment outcome in important ways. I have implemented hybrid agent-based computational models to investigate adaptive therapy, using either a single drug (cytotoxic or cytostatic), or two drugs (cytotoxic or cytostatic), simulating three different adaptive therapy protocols for treatment using a single drug (dose modulation, intermittent, dose-skipping), and seven different treatment protocols for treatment using two drugs: three dose modulation (DM) protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression), and four fixed-dose (FD) protocols (FD Cocktail Intermittent, FD Ping-Pong Intermittent, FD Cocktail Dose-Skipping, FD Ping-Pong Dose-Skipping). The results indicate a Goldilocks level of drug exposure to be optimum, with both too little and too much drug having adverse effects. Adaptive therapy works best under conditions of strong cellular competition, such as high fitness costs, high replacement rates, or high turnover. Clonal

competition is an important determinant of treatment outcome, and as such treatment using two drugs leads to more favorable outcome than treatment using a single drug. Switching drugs every treatment cycle (ping-pong) protocols work particularly well, as well as cocktail dose modulation, particularly when it is feasible to have a highly sensitive measurement of tumor burden. In general, overtreating seems to have adverse survival outcome, and triggering a treatment vacation, or stopping treatment sooner when the tumor is shrinking seems to work well.

DEDICATION

To humankind, my family, near and far, related by blood, or otherwise.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisor, Dr. Carlo Maley. This dissertation would not have seen the light of day without him. Throughout my PhD, he has been a constant source of inspiration, advice, and guidance, for all things, science and beyond. He has been a mentor, guide, and a friend. And more, he is family. I thank him for accepting me in his lab and letting me work on a project that has been a dream of mine. I have learnt so many things from him. Once again, it would be impossible to thank him enough. I plan on carrying his legacy forward, his visions and insights, in my own small way, if possible. I could not have asked for a better advisor.

I would like to give my heartfelt thanks to all members of my faculty committee, Luis Cisenros, Karen Anderson, Stephanie Forrest, and Carlo Maley. Thank you for being a beacon in my journey as a PhD student, and for all the intellectual discussions. I would also like to thank our collaborators, particularly Sandy Anderson, and Robert Noble. I would like to thank all the Maley lab members for the intellectual conversations.

I would like to take this opportunity to thank my family—my mom and dad, my fiancé Greer, and her family. Thank you Greer, for being in my life, for reminding me you believe in me as I navigate the hoops of the PhD process.

I would like to thank all my teachers, past and present, and to all people who have helped in some way to instill the spirit of scientific instinct and curiosity.

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CHAPTER 1

INTRODUCTION

Therapeutic resistance to existing anti-cancer drugs is a grave problem for cancer treatment (J. J. Cunningham, Gatenby, and Brown 2011) and, still remains, to this day, a formidable challenge that remains to be overcome for controlling or curing cancer. This is because the same drugs that once showed anti-cancer effects at the time therapy was initiated do not show the same effects later during treatment, leading to treatment failure, and eventually death (J. J. Cunningham, Gatenby, and Brown 2011). The primary goal of this dissertation has been to examine ways in which I can tackle the problem of therapeutic resistance. Indeed, if the problem of therapeutic resistance can be overcome, it would be possible to maintain indefinite control over cancer for the lifetime of the patient, converting cancer from an acute lethal disease that takes lives to a chronic disease that does not kill.

The one size fits all approach has typically been the mainstay for most cancer treatments up to this day, with the patient receiving the maximum tolerated dose of the cancer drug, with the goal being to eradicate the cancer (Gatenby 2009). While this hit hard and hit fast and hit often all-out attack approach might work for a tiny fraction of cancers, this approach also exacerbates the problem of drug resistance (Gatenby 2009). It turns out that some cancer cells, due to genetic (Wagle et al. 2011) or epigenetic phenomena, are able to withstand or "resist" the anti-cancer drug. These resistant cancer cells, even though possibly constituting a minority fraction of the overall cancer, would survive selectively, at the expense of the drug-sensitive cells, which are cancer cells on which the drug is able to exert the intended effect, such that the drug resistant cells

progressively become the more predominant cell type in the cancer, a phenomenon known in ecology as ‘competitive release’ (Zhang et al. 2017). Indeed, after competitive release has occurred, it would not be a stretch of imagination to see why most cancer treatments eventually lead to treatment failure.

The problem of drug resistance in oncology can be likened with that of pesticide resistance in agriculture (J. J. Cunningham, Gatenby, and Brown 2011; Gatenby 2009). Since the advent of the industrial revolution, there has been an explosion in the number of pesticides available in the market, and unrestrained use of these has led to the development of pesticide and herbicide resistance, which has grave consequences for the economy, leading to considerable losses in revenue. The farmers, growers, and pest managers have been able to tackle this problem of pesticide resistance by adopting a set of approaches to mange pests, collectively called Integrated Pest Management (IPM) (Barzman et al. 2015). Inspired by pest mangers, Gatenby et al has ushered in a new era of cancer treatment, which has been termed as adaptive therapy (Gatenby, Silva, Gillies, and Frieden 2009; Gatenby 2009; Gatenby, Brown, and Vincent 2009b).

So, the next question which arises is, what is adaptive therapy? Adaptive therapy can be viewed as tailoring treatment to the particular tumor, either by adjusting drug doses when the tumor grows, or by dosing a constant amount of the drug when the tumor is growing and withholding treatment when the tumor is shrinking. The idea being that, in the absence of the drug, the sensitive cells can dominate the competition with the resistant cells, levelling the playing field, and perhaps tilting the balance towards the sensitive cells, such that the tumor can still be controlled with the anti-cancer drug. As

such, adaptive therapy can be thought to be a form of personalized medicine, tailoring individual cancer treatment plan for the specific individual.

So, can the concept of adaptive therapy be borne out experimentally? As might have been expected, the answer is yes. Preclinical trials in mice with ovarian cancer has shown that a heuristic, shot in the dark algorithm of adjusting drug dosages work better than standard treatment (Gatenby, Silva, Gillies, and Frieden 2009). In a different preclinical trial in mice with breast cancer, the experimenters tested two different adaptive therapy protocols, dose-adjustment and dose-skipping, and came to the conclusion that the dose-adjustment works better than dose-skipping (Enriquez-Navas et al. 2016). In a clinical trial involving advanced stage metastatic castrate-resistant patients with prostate cancer, intermittent therapy, which could be conceived to be one form of adaptive therapy, was able to extend the median time to progression (TTP) to at least 27 months, relative to the 16.5 months median TTP that was observed in the contemporaneous cohort of patients that received standard treatment (Zhang et al. 2017).

Now, how do we come up with this individual treatment plan to curb resistance? The answer to this question lies in developing adaptive therapy treatment protocols that is best able to curb or combat resistance. In Chapter 2, I have investigated the simplest scenario possible, adaptive therapy using a single drug. In Chapter 3 and 4, I have investigated adaptive therapy treatment protocols with multiple drugs—specifically, two drugs.

Adaptive therapy with two drugs is considerably more challenging than adaptive therapy using a single drug. This is because, due to the law of combinatorial mathematics, the number of treatment parameters explode with each additional drug

(Thomas et al. 2022). Broadly speaking, two different drugs may be applied together such that they are at the same place at the same time, or the drugs could be applied successively ("rotated"), one after the other, such that only one drug is applied at any given time. It is unclear what is the best way to combine two drugs. As such, in Chapters 3 and 4, I have investigated multiple different ways to combine individual drugs together, with the goal of discovering the best ways to control or curb therapeutic resistance.

Moreover, anti-cancer drugs could have a cytotoxic mechanism of action, killing cells, or a cytostatic mechanism of action, preventing cellular division (Millar and Lynch 2003). However, very little research has been done to investigate how adaptive therapy using a single cytotoxic drug would compare to adaptive therapy using a single cytotoxic drug, or how adaptive therapy using two cytotoxic drugs would compare to adaptive therapy using two cytostatic drugs. In chapter 2, I have investigated adaptive therapy using a single cytotoxic, or a single cytostatic drug. In chapter 4, I have investigated adaptive therapy using two cytotoxic drugs, or two cytostatic drugs.

Furthermore, cancer is a loose term to describe a set of different diseases, all sharing certain hallmarks (Hanahan and Weinberg 2016, 2011), and each cancer can be conceived to be uniquely different from any other, and this concept extends not only for different cancer types, but also within the same cancer type. As such, cancers can be expected to have very different cellular kinetic parameters. An important point to mention is that, due to the sheer number of ways in which two drugs can be combined together, testing out all these different adaptive therapy protocols in mice is challenging, if not almost impossible, due to the time and the cost involved in the process. Thus, I have resorted to leverage the power of computational simulations to answer these

questions. The idea is that the best adaptive therapy protocols that are identified in these simulations could be tested first in preclinical experiments in mice with cancer, and if the results hold up, then these could further be tested in clinical trials in patients with cancer. In chapter 5, inspired by the spirit of translational research, I have undertaken to compare my computational modeling results to preclinical experiments conducted in mice with cancer. In Chapter 6, I write the conclusion, reiterating some key results obtained, and point out some possible avenue for further research in the field of adaptive therapy.

CHAPTER 2

IN SILICO INVESTIGATIONS OF ADAPTIVE THERAPY USING A SINGLE CYTOTOXIC OR A SINGLE CYTOSTATIC DRUG

Abstract

Adaptive therapy, as per the dose modulation, dose-skipping, or intermittent treatment protocol works well for treatment using a single cytotoxic drug, under a wide range of scenarios and parameter settings. In contrast, adaptive therapy works well only under a limited number of scenarios and parameter settings when using a single cytostatic drug. In general, adaptive therapy works best under conditions of higher fitness cost, higher replacement rate, higher turnover. Adaptive therapy works best when drug dosages are changed as soon as a change in tumor burden is detected. In general, it is better to pause treatment sooner than later, when the tumor is shrinking If the amount of drug used is too low, it is unable to control the sensitive cells and the tumor grows. However, if the drug dose is too high, it quickly selects for resistant cells and eventually the tumor grows out of control. However, there appears to be intermediate levels of dosing, which we call the minimum effective dose, which is able to control the sensitive cells but is not high enough to select for the resistant cells to grow out of control.

Introduction

Historically, treating cancer involves standard treatment (ST) at maximum tolerated dose (MTD) of a cancer drug. While this approach might work well for some cancer types, particularly ones with little heterogeneity, for most solid tumors this standard treatment eventually leads to an unresponsive tumor and consequent treatment failure instead of eradicating the cancer. This situation happens because under high doses

of the drug resistant cell clones survive and proliferate at the expense of sensitive cells, a phenomenon termed 'competitive release' (Enriquez-Navas, Wojtkowiak, and Gatenby 2015b). Yet there is a silver lining: adaptations for resistance normally entail a fitness cost. For instance, resistant MCF7Dox cells have a doubling time of 60 hours versus 40 hours for sensitive MCF7 cells in the absence of the drug, and in co-culture experiments the sensitive MCF7 cells outcompete resistant MCF7Dox cells (Gallaher et al. 2018). Adaptive therapy utilizes this principle of fitness penalty incurred by resistant cells in absence of the drug in order to maintain long-term control over the tumor (Gatenby 2009; Enriquez-Navas, Wojtkowiak, and Gatenby 2015b; Gallaher et al. 2018; Gatenby, Brown, and Vincent 2009b; Gatenby, Silva, Gillies, and Frieden 2009; Enriquez-Navas et al. 2016; Zhang et al. 2017; J. West et al. 2020; J. B. West et al. 2019b; Ibrahim-Hashim et al. 2017; Bacevic et al. 2017; Buhler et al. 2021; A. Araujo et al. 2021; Brady-Nicholls et al. 2020; J. Cunningham et al. 2020; Hansen and Read 2020b; Thomas et al. 2022). It has been shown by Gatenby and colleagues that robust cancer control is possible with adaptive therapy as long as there is a substantial fitness cost to resistance. Multiple theoretical and mathematical models of adaptive therapy has been formulated. A vast array of models calibrate models to fit experimental data (J. West et al. 2020; Strobl et al. 2021; J. B. West et al. 2019b).

Preclinical experiments in mice with breast cancer demonstrated the superiority of dose modulation protocol, which involves adjusting drug dosages in response to changes in tumor burden over dose-skipping protocol, which involves administration of fixed-dosage of the drug if the tumor grows (Enriquez-Navas et al. 2016). Stable tumor burden was maintained in mice with breast cancer by a heuristic dose adjustment treatment

protocol (Gatenby, Silva, Gillies, and Frieden 2009). Intermittent therapy trials, in which drug is administered until tumor burden shrinks to a fraction of the baseline followed by withholding drug until tumor burden increases to the baseline, in patients with prostate cancer resulted in an increase in median time to progression by at least 27 months compared to a contemporaneous cohort of patients (Zhang et al. 2017). Preclinical adaptive therapy experiments were conducted in mice with breast cancer using paclitaxel (Enriquez-Navas et al. 2016; Gatenby, Silva, Gillies, and Frieden 2009), or in mice with ovarian cancer using carboplatin (Gatenby, Silva, Gillies, and Frieden 2009). Both these drugs are cytotoxic in their mode of action. Previous adaptive therapy experiments have also been carried out using a cytotoxic drug (Enriquez-Navas et al. 2016). In contrast, the clinical trial carried out in prostate cancer patients using intermittent therapy used abiraterone (plus prednisone) (Zhang et al. 2017), a hormone therapy drug with a cytostatic mode of action. The main distinction is that cytotoxic drugs work by killing cancer cells, while the mode of action of cytostatic drugs is the inhibition of tumor growth by suppressing cell division (Millar and Lynch 2003).

There are different variations and parameters for different types of adaptive therapy. While the dose-modulation protocol involves adjusting drug dosages based on changes in tumor burden since the previous treatment cycle, the dose-skipping or intermittent treatment protocols typically involve administering fixed dosages of the drugs every treatment cycle. Also, due to wide variations possible in cell kinetics, multiple scenarios exist. In addition to tumor growth kinetics, each adaptive therapy treatment protocol involves its own specific set of treatment parameters. Furthermore, because cytotoxic and cytostatic drugs vary fundamentally in their mode of action,

survival outcomes could reasonably be expected to vary widely, thus designing adaptive therapy protocols that are best for a given situation and circumstance can be a challenging task. In this article, we sought to investigate which treatment protocol would be the best or most optimum for given situations or circumstances, on a case-by-case basis. We investigate three types of adaptive therapy treatment protocols, namely, dose modulation, dose-skipping, and intermittent therapy, as well as standard treatment at maximum tolerated dose under a wide range of conditions, with the goal of finding the best or the most optimum treatment protocol.

Materials and Methods

We modified the agent-based hybrid model we previously published (Thomas et al. 2022) to simulate adaptive therapy using a single drug and correspondingly extended the Hybrid Automata Library (HAL) agent-based modeling framework (Bravo et al. 2020). Drug diffusion is modeled by solving the diffusion partial differential equations (Bravo et al. 2020). The tumor consists of two different cell types: the sensitive cells and the resistant cells, which are situated on a 2-dimensional 100 by 100 square lattice. Each cell is modeled as an on-lattice agent, such that they occupy the lattice unit in which they are located and are not free to move around. Each cell, at every time step, could die or divide. As a first approximation, we assume that cytotoxic drugs only work by killing cells, and cytostatic drugs only work by inhibiting cell division. Thus, when cytotoxic drugs are used, sensitive cells die as a function of drug concentration as well as due to the background cell death rate, while resistant cells die only because of the background cell death rate. When cytostatic drugs are used, cells die only due to a background cell death rate (apoptosis rate) which is the same for all cell types. If a cell manages to survive, it

could either divide or not, depending on the rate of cell division for that particular cell type. When a cell is committed to dividing, it could either divide so that a new cell occupies one of the available spaces in its Moore neighborhood, if any, or divide by replacing a neighbor, or do nothing if no empty space is available and cells are not allowed to replace a neighbor. Whether or not cells can replace a neighbor is governed by the replacement parameter. Setting the replacement parameter to zero enforces contact inhibition in the model and cells are not able to replace a neighbor while setting it to one allows dividing cells to replace a neighbor every time a cell is committed to dividing and an empty space is not available. Setting the replacement parameter to intermediate levels, such as 0.5, entails that cells committed to dividing would replace a neighbor 50% of the time, and not be able to replace a neighbor 50% of the time. When a cell divides, a daughter cell is created of the same type as the parent and is situated in one of the neighboring lattices. Both the daughter cells (the parent cell now having divided is considered one of the daughter cells) could mutate when cells divide. Whether or not the daughter cells would mutate after cell division is governed by a set of mutation parameters. We allow for bidirectional mutation in the model, meaning that a sensitive cell could either mutate to become a resistant cell, or not, as well as a resistant cell could mutate to become a sensitive cell, or not, at every time-step when cell division occurs. For the default parameter values, we set the mutation rate from sensitive to resistant cells, and vice-versa, to 10^{-3} per cell division, in order to account for the unrealistically low number of cells in our model. We have incorporated fitness cost in our model by choosing higher division rates for sensitive cells and lower division rates for resistant cells. Thus at any time step the division probability of sensitive cells > the division

probability of resistant cells. When cytostatic drugs are being used, the sensitive cells undergo a decrease in their division rates as a function of the drug concentration, while the division rates for the resistant cells remain unaffected. At every time-step after the start of treatment drug diffusion is modeled by using the alternating direction implicit (ADI) method (Bravo et al. 2020).

The treatment protocols being considered are the three different adaptive therapy protocols: dose modulation, dose-skipping, and intermittent; as well as standard treatment, which serves as the control. All adaptive therapy protocols involve monitoring the tumor every three days, and treatment starts as soon as the tumor burden equals or exceeds 50% of the carrying capacity, which is the total number of cancer cells that can be accommodated in the grid.

Dose Modulation

The dose modulation protocols have two primary parameters: Delta Tumor, which is the percentage by which the tumor burden must change relative to the previous treatment cycle, in order to trigger a change in drug dose and, Delta Dose, which is the percentage by which the drug dose is changed relative to the previous treatment cycle. In this treatment the drug dosage is increased when the tumor has grown by more than Delta Tumor, or decreased when the tumor has shrunk by more than Delta Tumor, or maintained at the same dosage level as the previous treatment cycle when the tumor is either growing or shrinking by less than the Delta Tumor threshold. The drug dosage is capped so it never exceeds the maximum tolerated dose at which treatment is initiated, and never decreases beyond the minimum drug dose, which represents the amount below which the drug has no physiologic effect (and a dosage that in fact cannot be formulated

in laboratory settings). In addition, if the absolute tumor burden ever exceeds the maximum of what has been recorded so far since initiation of treatment, drug dosage is increased by Delta Dose. Furthermore, a treatment vacation is triggered when the tumor burden falls to, or below a certain threshold ("stop dosing"), such that no drug is administered for that treatment cycle.

Dose-Skipping

In contrast to the dose modulation protocol, the dose-skipping protocol involves administering a constant amount of the drug (fixed drug dosage). Drug is administered at that fixed level only when the tumor is growing above the Delta Tumor threshold, in all other cases no drug is administered (hence the treatment is "skipped" for that treatment cycle).

Intermittent

The intermittent protocol involves monitoring the absolute tumor burden every treatment cycle. Treatment starts as soon as the tumor burden equals or exceeds 50% of the carrying capacity, which is considered to be 100% of the baseline level. A fixed-dose of the drug is administered at every treatment cycle until the tumor shrinks to 50% or more of the baseline level, at which point no drug is administered in any treatment cycle until the tumor burden increases to 100% of the baseline, and so on and so forth. The intermittent protocol has a key parameter: at what tumor burden should the treatment be stopped when the tumor is shrinking, in order that the tumor may be allowed to climb back up to the baseline value at which treatment was initiated previously. As mentioned above, this "stop dosing" threshold is chosen to be 50% of the baseline for the default parameter value.

The complete description of the model using the standard overview design details (ODD) format for describing agent-based models (Grimm et al. 2010) can be found in (Thomas et al. 2022) with the following changes:

In section 2.2 (Entities, State Variables, and Scales), we have considered two different cell types: sensitive, and resistant to account for treatment using either a single cytotoxic, or a single cytostatic drug.

In section 2.4.11 (Observation), we have made some modification to our criterion for progression. The modified survival criterion is as follows: If the tumor burden equaled or exceeded 97% of the carrying capacity at any point after initiation of therapy, or the rolling average of the total number of resistant cells over 500 time-steps equaled or exceeded 50% of the carrying capacity, then the particular run is scored as "Progressed" and the time at which progression takes place after therapy initiation is noted.

In section 2.5 (Initialization), instead of considering 4 different cell types to account for 2 drugs, in the initial tumor seed, we now consider 2 different cell types: sensitive and resistant to account for the cell types that are either sensitive, or resistant, to the single drug studied here.

In section 2.7.1 (Cell Death), for treatment with a single cytotoxic drug, the equation for probability of cell death is as follows: Probability of cell death per hour=background death probability per hour+S1*[Drug1]*Ψ1, where S1 is the binary indicator variable for the cell's sensitivity to drug 1,[Drug1] being the concentration of drug 1 (non-negative real values), and Ψ1 is the drug potency (non-negative real values), quantified as the probability of cell death per unit drug concentration per hour. For

treatment using a single cytostatic drug, the equation for probability of cell death is as follows: Probability of cell death per hour=background death probability per hour.

In section 2.7.2 (Cell Division), the cell division rates for the sensitive cell is 0.06 per hour, the cell division rate for the resistant cell is 0.02 per hour. The division probabilities can now be arranged in the following descending order:sensitive cells > resistant cells. For treatment with a single cytostatic drug, probability of cell division per hour=background division probability per hour-S1*[Drug1]*Ψ1, where S1 is the binary indicator variable for the cell's sensitivity to drug 1, [Drug1] is the concentration of drug 1 (non-negative real values), and Ψ1 is the drug potency (non-negative real values), quantified as the probability of inhibition in cell division per unit drug concentration per hour.

Section 2.7.4 (Mutation): The default value for the mutation rate parameter is 10-3 per cell division, to account for transition from sensitive cell type to resistant cell type, and vice-versa.

In section 2.7.6 (Drug Protocols): The treatment protocols are described as follows:

Standard Treatment (ST): Drug 1 was administered at maximum tolerated dose (MTD) once every 24 hours for the entire duration of the simulation (Fig. 2.1).

Dose Modulation: Treatment started at MTD with Drug 1, and dosage of the drug was adjusted according to the dose modulation adaptive therapy protocol, parameterized by Delta Tumor, and Delta Dose (Fig. 2.1). This treatment protocol was equivalent to the standard dose modulation adaptive therapy protocol (AT-1) from previous experiments.

Dose-Skipping: Drug 1 was administered at a fixed-dose that was set at 75% of MTD. If the tumor grew by more than Delta Tumor since its last measurement, or if the tumor burden exceeded its previous maximum size, the drug was applied or else, the dose was skipped. (Fig. 2.1). This is equivalent to AT-2 protocol from previous experiments.

Intermittent: Treatment started at 75% of the MTD using Drug 1, drug being administered once every 24 hours. Treatment was stopped when a shrinkage in tumor burden by at least 50% relative to the tumor burden at which therapy was initiated is detected, and therapy was restarted when the tumor burden equaled or exceeded 100% of the value at which therapy was initiated (Fig. 2.1). This protocol is equivalent to the prostate cancer clinical trial carried out in cancer patients with the drug abiraterone.

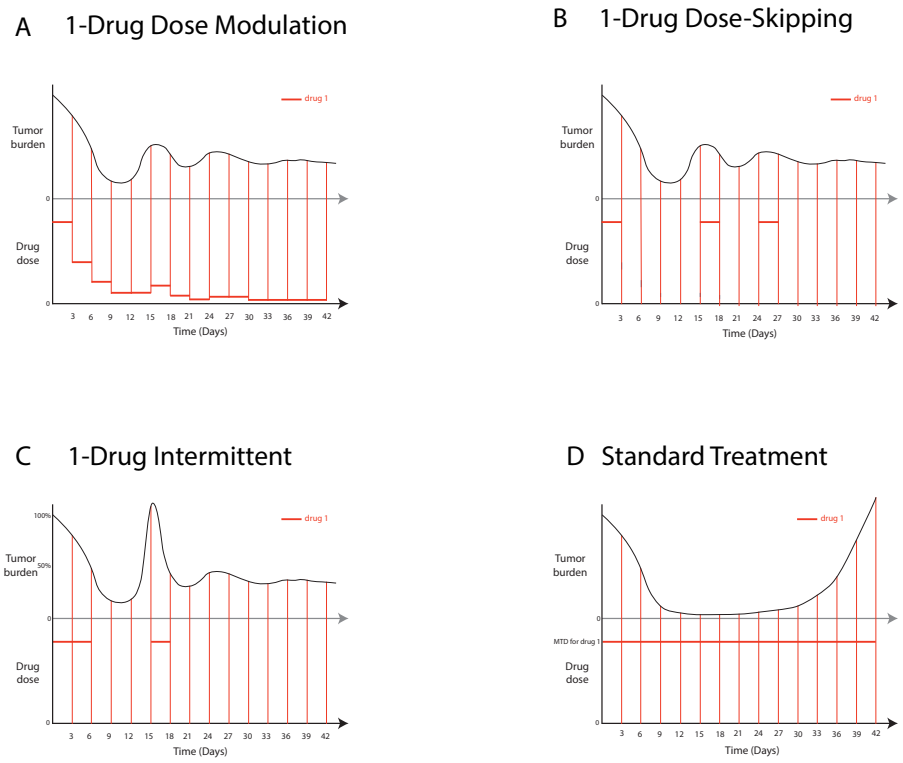

Figure 2.1: Single-drug adaptive therapy protocols using a single cytotoxic, or a single cytostatic drug. Schematic depicting dose modulation protocol (Fig. 2.1A), dose-skipping (Fig. 2.1B), intermittent (Fig. 2.1C), and standard treatment (Fig. 2.1D).

Results

Cytotoxic and Cytostatic Therapies

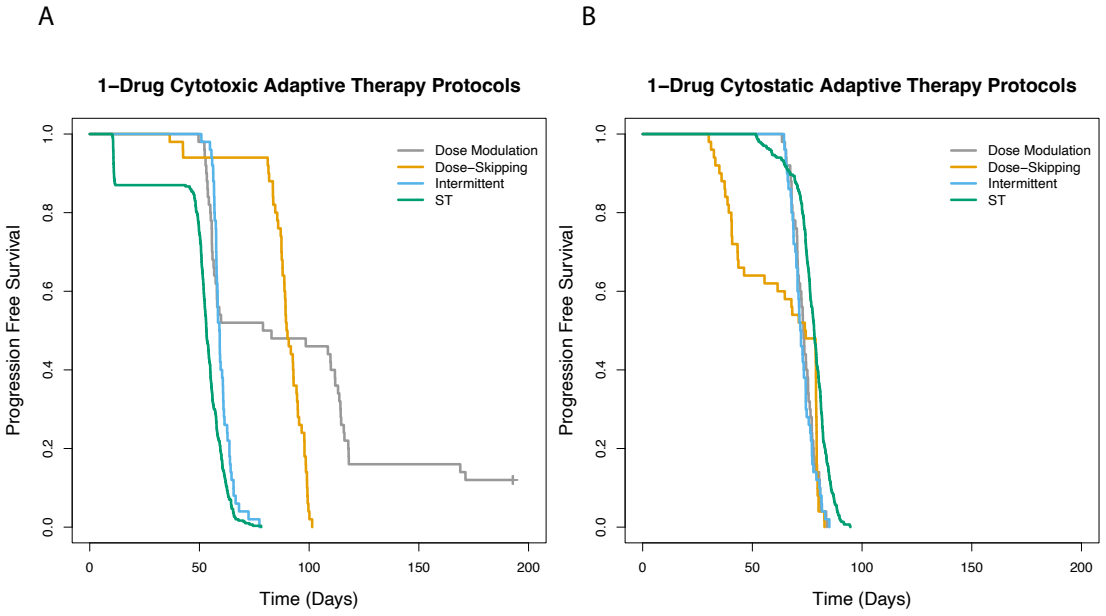

Figure 2.2: Adaptive therapy using a single cytotoxic or a single cytostatic drug. Single-drug adaptive therapy protocols comparing standard treatment (ST) versus three different adaptive therapy protocols, dose modulation, dose-skipping, and intermittent using a single cytotoxic drug (Fig. 2.2A), or a single cytostatic drug (Fig. 2.2B).

For treatment using a single cytotoxic drug, all the protocols, that is, dose modulation, dose-skipping, and intermittent work well, increasing TTP relative to standard treatment (Fig. 2.2A, Table 2.1), although the effect size as measured by the hazard ratio was small for intermittent (Table S1). For treatment using a single cytostatic drug, none of the protocols, that is, dose-modulation, intermittent, or dose-skipping is able to increase TTP relative to standard treatment (Fig. 2.2B, Table S1).

Table 2.1: Adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				

Default	Standard Treatment	0.18	0.11-0.27	<0.001
Dose Skipping (cytotoxic)				
Default	Standard Treatment	0.01	0.003-0.037	<0.001
Intermittent (cytotoxic)				
Default	Standard Treatment	0.51	0.38-0.69	<0.001
Dose Modulation (cytostatic)				
Default	Standard Treatment	2.4	1.7-3.2	<0.001
Dose Skipping (cytostatic)				
Default	Standard Treatment	2.2	1.6-3.0	<0.001
Intermittent (cytostatic)				
Default	Standard Treatment	2.6	1.9-3.5	<0.001

The Effect of Fitness Costs of Resistance

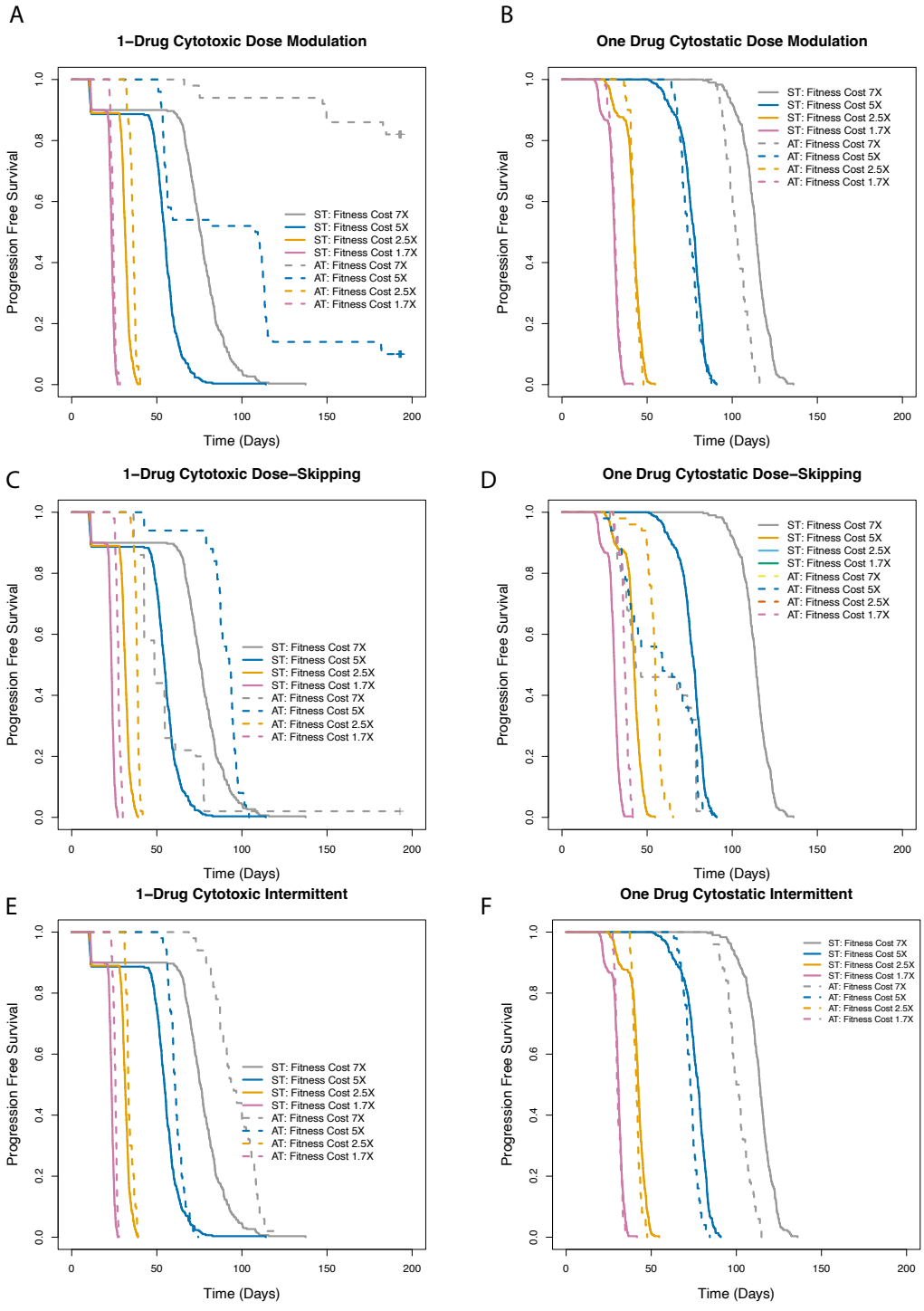

Figure 2.3: Effect of fitness cost parameter on the outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug. Survival outcome for treatment as per the dose modulation protocol (Fig. 2.3A, Fig. 2.3B), dose-skipping (Fig. 2.3C, Fig. 2.3D), or intermittent (Fig. 2.3E, Fig. 2.3F) under fitness cost of 1.7-fold, 2.5-fold, 5-fold, or 7-fold

relative to standard treatment for treatment using either a single cytotoxic (Fig. 2.3A, 2.3C, 2.3E), or a single cytostatic drug (Fig. 2.3B, 2.3D, 2.3F).

Fitness cost incurred by resistant cells, as manifested in longer doubling times relative to sensitive cells in the absence of the drug, plays an important role in determining the outcome of both cytotoxic as well as cytostatic single-drug adaptive therapy. In general, adaptive therapy using a single cytotoxic drug works better than standard treatment at fitness cost of 1.7-fold, 2.5-fold, 5-fold, or 7-fold, increasing TTP, for treatment as per the dose modulation, intermittent, and dose-skipping protocol (Fig. 2.3, Table 2.2). The exception to this trend is adaptive therapy is not working well relative to the standard treatment at a fitness cost of 7-fold for treatment as per the dose-skipping protocol (Fig. 2.3C, Table 2.2). Moreover, in general, adaptive therapy treatment protocols under conditions of higher fitness cost leads to improved survival outcome relative to treatment under conditions of lower fitness cost. Thus, adaptive therapy at 7-fold fitness cost leads to better survival outcome than treatment under 5-fold fitness cost for dose-modulation as well as the intermittent treatment protocol (Table 2.2). However, for treatment with a cytostatic drug, none of the protocols tested here resulted in increased TTP relative to standard treatment at any of the fitness cost values tested here except dose-skipping protocol under fitness cost of 1.7-fold (Fig. 2.3B,2.3D,2.3F, Table 2.2).

Moreover, in general, higher fitness cost (such as 7-fold fitness cost) translated to an improvement in survival outcome relative to lower fitness cost (such as 5-fold) (Table 2.3).. Thus, for treatment using a single cytotoxic drug, as per the dose modulation protocol, as well as the intermittent resulted in increased TTP relative to standard

treatment for all the fitness cost values tested (Fig. 2.3, Table 2.2, all $p < 0.001$). For treatment with the dose-skipping protocol using a cytotoxic drug (Fig. 3C; Table S1), all fitness values except the 7-fold fitness cost results in significant increase in TTP relative to standard treatment. For the intermittent protocol using a cytotoxic drug (Fig. 2.3E), all values of fitness cost tested here resulted in significantly increased TTP relative to standard treatment (Fig. 2.3A; Table 2.2). We observe increased TTP comparing higher fitness cost to lower fitness costs (Table 2.2). As an exception to this general trend, however, we observe increased TTP under 5-fold fitness cost compared to 7-fold fitness cost with dose-skipping using a cytotoxic drug.

Table 2.2: Effect of fitness cost parameter on the outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.59	0.44–0.81	< 0.001
2.5-fold fitness cost	Standard Treatment	0.30	0.22-0.41	<0.001
5-fold fitness cost	Standard Treatment	0.21	0.14-0.31	<0.001
7-fold fitness cost	Standard Treatment	0.01	0.003-0.037	<0.001
7-fold fitness cost	5-fold fitness cost	0.08	0.04-0.16	<0.001
Dose Skipping (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.08	0.05-0.13	<0.001

2.5-fold fitness cost	Standard Treatment	0.11	0.07-0.16	<0.001
5-fold fitness cost	Standard Treatment	0.11	0.07-0.16	<0.001
7-fold fitness cost	Standard Treatment	2.7	2.0-3.8	<0.001
5-fold fitness cost	7-fold fitness cost	0.17	0.11-0.28	<0.001
Intermittent (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.31	0.22-0.42	<0.001
2.5-fold fitness cost	Standard Treatment	0.50	0.37-0.68	<0.001
5-fold fitness cost	Standard Treatment	0.55	0.41-0.74	<0.001
7-fold fitness cost	Standard Treatment	0.34	0.25-0.46	<0.001
7-fold fitness cost	5-fold fitness cost	0.01	0.003-0.040	<0.001
Dose Modulation (cytostatic)				
1.7-fold fitness cost	Standard Treatment			Not Significant
2.5-fold fitness cost	Standard Treatment			Not Significant
5-fold fitness cost	Standard Treatment			Not Significant
7-fold fitness cost	Standard Treatment	4.9	3.5-6.8	<0.001
2.5 -fold fitness cost	1.7-fold fitness cost	0.01	0.002-0.045	<0.001

Dose Skipping (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.16	0.11-0.23	<0.001
2.5-fold fitness cost	Standard Treatment	0.06	0.03-0.10	<0.001
5-fold fitness cost	Standard Treatment	2.1	1.6-2.9	<0.001
7-fold fitness cost	Standard Treatment			Not Significant
Intermittent (cytostatic)				
1.7-fold fitness cost	Standard Treatment			Not Significant
2.5-fold fitness cost	Standard Treatment	1.6	1.2-2.2	<0.01
5-fold fitness cost	Standard Treatment	2.2	1.6-3.0	<0.001
7-fold fitness cost	Standard Treatment	5.9	4.2-8.3	<0.001

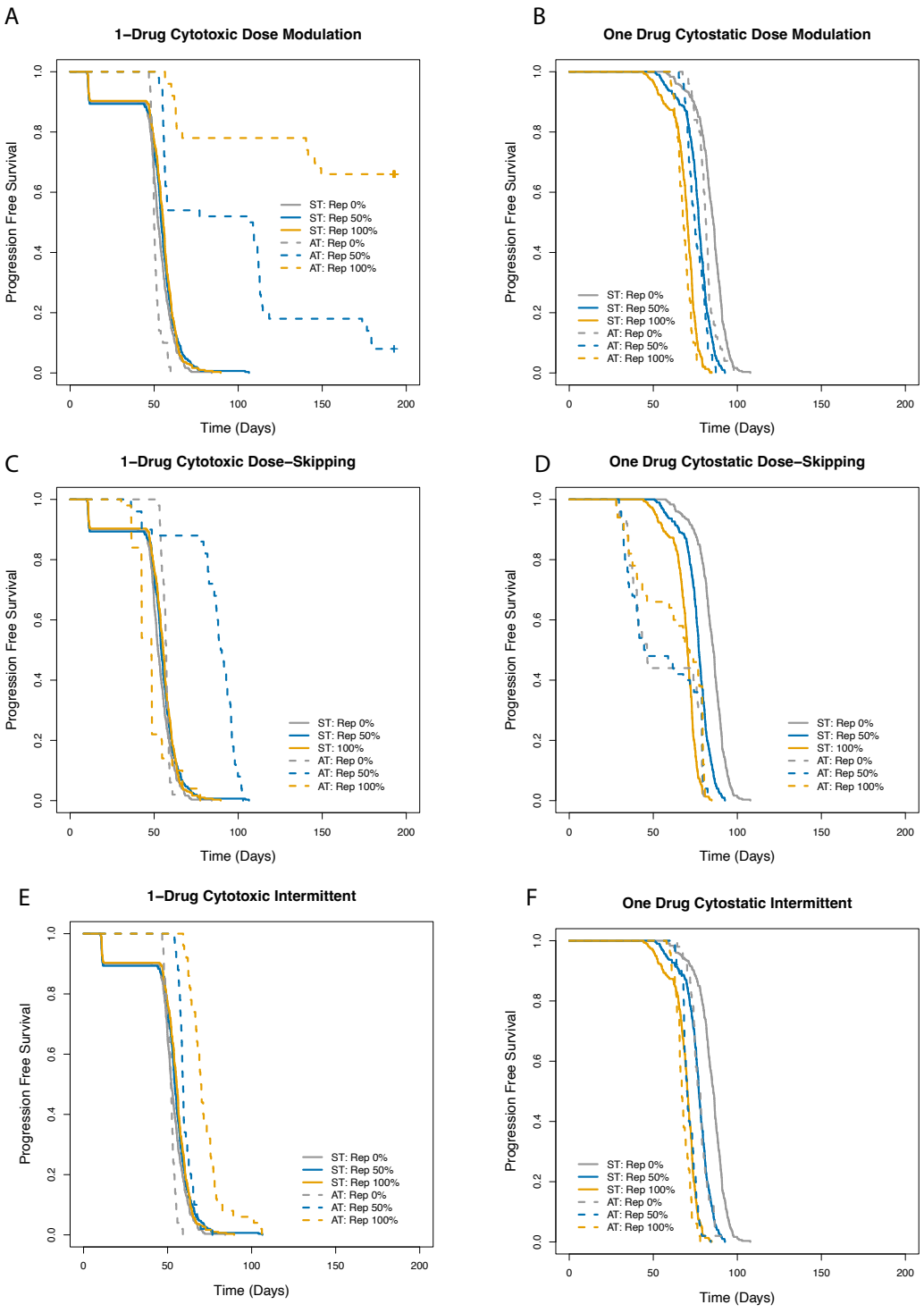

Figure 2.4: Effect of replacement parameter on outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug. Treatment as per the dose modulation protocol (Fig. 2.4A, 2.4B), dose-skipping protocol (Fig. 2.4C, Fig. 2.4D), or intermittent (Fig. 2.4E,Fig. 2.4F), relative to standard treatment under conditions of 0%, 50%, or

100% replacement using either a single cytotoxic (Fig. 2.4A,2.4C, 2.4E), or a single cytostatic drug (Fig. 2.4B, Fig. 2.4D, Fig. 2.4F).

Cell Competition

The relationship between cell crowding, cell death and cell proliferation and direct cell competition is unknown. We encapsulated this complexity in a parameter (Cell Replacement) that specifies the likelihood that a cell can replace its neighbor if there are no empty spaces in its immediate neighborhood when it tries to divide.

In general, for treatment using a single cytotoxic drug, under conditions of 50% or 100% replacement rate, every adaptive therapy protocol works well, increasing TTP relative to standard treatment (Fig. 2.4, Table 2.3), an exception being treatment as per the dose-skipping protocol under conditions of 100% replacement (Fig. 2.4C, Table 2.3). However, under conditions of 0% replacement rate, no improvement in survival outcome was observed relative to standard treatment for any of the adaptive therapy protocols tested here. In general, adaptive therapy under conditions of higher replacement rates (more direct competition) results in improved survival outcome relative to treatment under conditions of lower replacement rates (Tables 2.3). Thus, survival outcome is better at 50% replacement rate relative to 0% replacement rate, or at 100% replacement relative to 50% for all of the adaptive therapy protocols using a single cytotoxic drug (Table 2.3), an exception being survival outcome is better under 50% relative to 100% replacement rate for treatment as per the dose-skipping protocol (Fig. 2.4, Table 2.3). In contrast, unlike treatment using a single cytotoxic drug, no improvement in survival outcome relative to standard treatment was observed when treated using a single

cytostatic drug for any of the adaptive therapy protocols (Fig. 2.4B, 2.4D, 2.4F) tested here.

In general, higher replacement probabilities lead to better survival outcome relative to lower replacement probabilities. We observe increased TTP relative to standard treatment under conditions of higher replacement for treatment as per the dose modulation protocol using a cytotoxic drug (Table S2). An exception, however, to this general trend is treatment using a single cytotoxic drug using the dose-skipping protocol leads to increased TTP relative to standard treatment under conditions of 100% replacement versus 50% replacement (Fig. 2.4C, Table 2.3). In contrast, treatment with cytostatic drugs (Fig. 2.4B, 2.4D, 2.4F) do not result in increased TTP relative to standard treatment under any of the replacement conditions tested (Fig. 2.4B, 2.4D, 2.4F).

Table 2.3: Effect of replacement parameter on outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				
0% replacement	Standard Treatment	1.9	1.4-2.6	<0.001
50% replacement	Standard Treatment	0.17	0.11-0.27	<0.001
100% replacement	Standard Treatment	0.05	0.03-0.10	<0.001
50% replacement	0% replacement	0.12	0.07-0.20	<0.001
100% replacement	50% replacement	0.18	0.10-0.32	<0.001

Dose Skipping (cytotoxic)				
0% replacement	Standard Treatment			Not Significant
50% replacement	Standard Treatment	0.16	0.11-0.23	<0.001
100% replacement	Standard Treatment	1.7	1.3-2.3	<0.001
50% replacement	0% replacement	0.04	0.02-0.11	<0.001
100% replacement	50% replacement	24.8	10.0-61.4	<0.001
Intermittent (cytotoxic)				
0% replacement	Standard Treatment	1.6	1.2-2.2	<0.01
50% replacement	Standard Treatment	0.60	0.45-0.81	<0.001
100% replacement	Standard Treatment	0.22	0.16-0.31	<0.001
50% replacement	0% replacement	0.08	0.04-0.14	<0.001
100% replacement	50% replacement	0.18	0.11-0.29	<0.001
Dose Modulation (cytostatic)				
0% replacement	Standard Treatment	1.8	1.3-2.4	<0.001
50% replacement	Standard Treatment	1.4	1.0-1.9	<0.05
100% replacement	Standard Treatment	1.4	1.0-1.8	<0.05

Dose Skipping (cytostatic)				
0% replacement	Standard Treatment	11.3	7.7-16.6	<0.001
50% replacement	Standard Treatment	2.4	1.8-3.3	<0.001
100% replacement	Standard Treatment	0.60	0.43-0.82	<0.01
Intermittent (cytostatic)				
0% replacement	Standard Treatment	3.7	2.7-5.1	<0.001
50% replacement	Standard Treatment	3.4	2.5-4.7	<0.001
100% replacement	Standard Treatment	1.7	1.2-2.3	<0.001

Cell Turnover

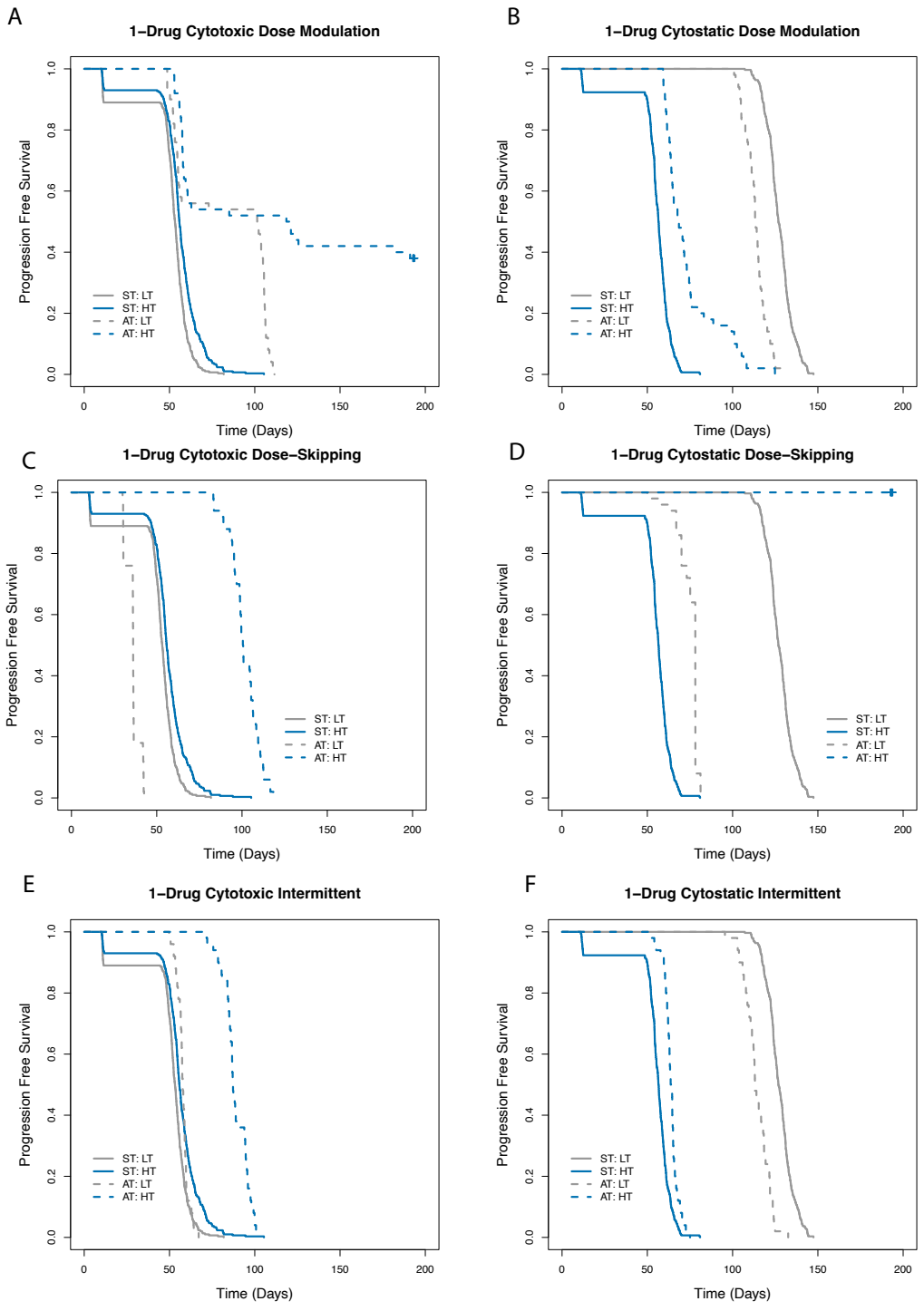

Figure 2.5: Effect of turnover on outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug. Survival outcome for treatment as per the dose modulation protocol (Fig. 2.5A, Fig. 2.5B), dose-skipping protocol (Fig. 2.5C, Fig. 2.5D), or intermittent (Fig. 2.5E, Fig. 2.5F) using a single cytotoxic (Fig. 2.5A, 2.5C, 2.5E) or a

single cytostatic drug (Fig. 2.5B, 2.5D, 2.5F) under conditions of low turnover (LT) or high turnover (HT), relative to standard treatment under those conditions.

For treatment with a single cytotoxic drug, under low turnover conditions, dose modulation (Fig. 2.5A) and intermittent treatment protocols (Fig. 2.5E) results in increased TTP relative to standard treatment but no increased TTP is observed for dose-skipping (Fig. 2.5C). For treatment with a single cytostatic drug, under conditions of low turnover, none of the protocols leads to increased TTP relative to standard treatment (Fig, 2.5B, 2.5D, 2.5F), standard treatment working well under these conditions. However, under conditions of high turnover, when treated with a single cytotoxic drug (Fig. 2.5A, 2.5C, 2.5E), or a single cytostatic drug (Fig. 2.5B, 2.5D, 2.5F), every adaptive therapy protocol tested here, that is, dose modulation (Fig. 2.5A, 2.5B), dose-skipping (Fig. 2.5C,2.5D), and intermittent (Fig. 2.5E, 2.5F) treatment leads to increased TTP relative to standard treatment. In general, for treatment with a single cytotoxic drug, we observed improved survival outcomes under conditions of high turnover relative to conditions of low turnover (Table 2.4).

Table 2.4: Effect of turnover on outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				
Low Turnover	Standard Treatment	0.17	0.11-0.26	<0.001
High Turnover	Standard Treatment	0.17	0.11-0.27	<0.001

High Turnover	Low Turnover	0.30	0.18-0.50	<0.001
Dose Skipping (cytotoxic)				
Low Turnover	Standard Treatment	14.6	9.1-23.3	<0.001
High Turnover	Standard Treatment	0.06	0.04-0.10	<0.001
High Turnover	Low Turnover	~0		
Intermittent (cytotoxic)				
Low Turnover	Standard Treatment	0.63	0.47-0.85	<0.01
High Turnover	Standard Treatment	0.13	0.09-0.19	<0.001
High Turnover	Low Turnover	~0		
Dose Modulation (cytostatic)				
Low Turnover	Standard Treatment	8.5	6.0-11.9	<0.001
High Turnover	Standard Treatment	0.19	0.13-0.27	<0.001
High Turnover	Low Turnover	6.4	4.1-10.1	<0.001
Dose Skipping (cytostatic)				
Low Turnover	Standard Treatment			Not Significant
High Turnover	Standard Treatment	~0		

High Turnover	Low Turnover	~0		
Intermittent (cytostatic)				
Low Turnover	Standard Treatment	6.7	4.8-9.3	<0.001
High Turnover	Standard Treatment	0.40	0.29-0.54	<0.001
Low Turnover	High Turnover	~0		

When to Adjust the Dose of the Drug

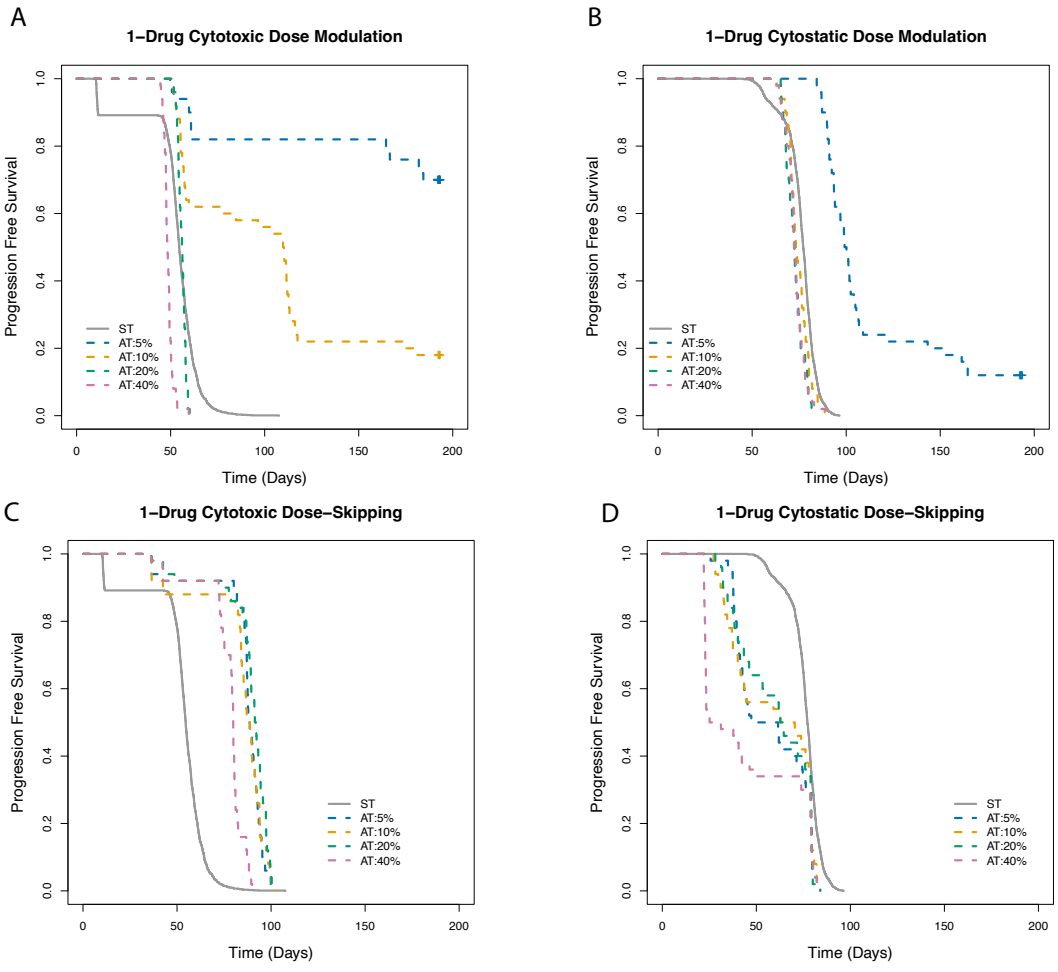

Figure 2.6: Effect of the delta tumor parameter on determining the outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug. Survival outcome comparing dose modulation treatment protocol with Delta Tumor=5%, 10%, 20%, or 40% using a single cytotoxic (Fig. 2.6A), or a single cytostatic drug (Fig. 2.6B) relative to standard treatment. Survival outcome comparing dose-skipping treatment protocol with Delta Tumor=5%, 10%, 20%, or 40% using a single cytotoxic (Fig. 2.6C), or a single cytostatic drug (Fig. 2.6D).

The dose modulation protocols have two primary parameters: Delta Tumor, which is the amount the tumor burden must change in order to trigger a change in drug dose and, Delta Dose, which is the amount by which the dose is changed.

For treatment using a single cytotoxic drug, as per the dose-modulation protocol, delta tumor=5%, or delta tumor=10% leads to increased TTP relative to standard treatment (Fig. 2.6A, Table 2.5), while treatment as per the dose-skipping protocol works well for all values of delta tumor tested here, that is, delta tumor=5%, 10%, 20%, or 40% (Fig. 2.6C, Table 2.5). However, only treatment as per the dose modulation protocol with delta tumor=5% increased TTP relative to standard treatment (Fig. 2.6B, Table 2.5). In general, the lower the value of delta tumor, the better is the survival outcome. Thus, delta tumor=5% leads to better survival outcome relative to delta tumor=10% for treatment as per the dose modulation protocol when using a single cytotoxic, or a single cytostatic drug (Table 2.5). These results indicate that treatment as per the dose modulation protocol works best if dose is adjusted as soon as a change in tumor burden is detected. In practice this will likely be limited by the sensitivity of the tumor burden assay. Note that using a small Delta Tumor value allows the dose modulation protocol to be effective even with a cytostatic drug (Fig. 2.6B, Table 2.5).

Table 2.5: Effect of the delta tumor parameter on determining the outcome of adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				
Delta Tumor=5%	Standard Treatment	0.03	0.02-0.06	<0.001
Delta Tumor=10%	Standard Treatment	0.11	0.07-0.17	<0.001
Delta Tumor=20%	Standard Treatment			Not Significant
Delta Tumor=40%	Standard Treatment	4.4	3.3-6.0	<0.001
Delta Tumor=5%	Delta Tumor-10%	0.21	0.11-0.38	<0.001
Dose Skipping (cytotoxic)				
Delta Tumor=5%	Standard Treatment	0.12	0.09-0.17	<0.001
Delta Tumor=10%	Standard Treatment	0.13	0.10-0.19	<0.001
Delta Tumor=20%	Standard Treatment	0.11	0.08-0.16	<0.001
Delta Tumor=40%	Standard Treatment	0.19	0.14-0.26	<0.001
Dose Modulation (cytostatic)				
Delta Tumor=5%	Standard Treatment	0.06	0.04-0.10	<0.001
Delta Tumor=10%	Standard Treatment	1.5	1.1-2.0	<0.01
Delta Tumor=20%	Standard Treatment	2.3	1.8-3.1	<0.001

Delta Tumor=40%	Standard Treatment	2.1	1.6-2.8	<0.001
Delta Tumor=5%	Delta Tumor=10%	0.02	0.006-0.049	<0.001
Dose Skipping (cytostatic)				
Delta Tumor=5%	Standard Treatment	2.5	1.8-3.3	<0.001
Delta Tumor=10%	Standard Treatment	2.0	1.5-2.6	<0.001
Delta Tumor=20%	Standard Treatment	2.3	1.7-3.0	<0.001
Delta Tumor=40%	Standard Treatment	2.7	2.1-3.6	<0.001

How much to change the dose for the dose modulation protocols

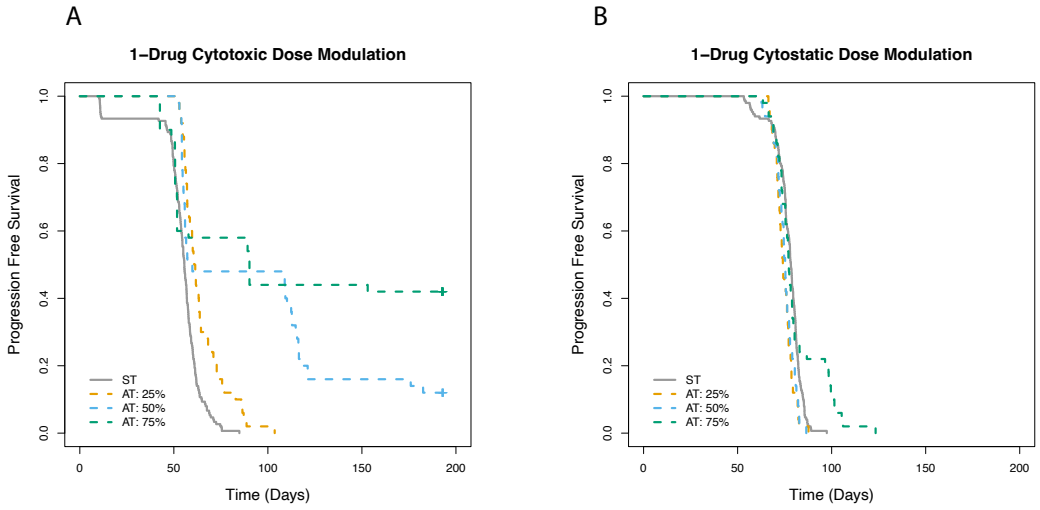

Figure 2.7: Effect of the delta dose parameter on determining the outcome of dose modulation adaptive therapy using a single cytotoxic or a single cytostatic drug. Survival outcome for treatment as per the dose modulation protocol with Delta Dose=25%, 50%, or 75% relative to standard treatment using a single cytotoxic drug (Fig. 2.7A), or a single cytostatic drug (Fig. 2.7B).

The dose modulation protocols have two primary parameters: Delta Tumor, which is the amount the tumor burden must change in order to trigger a change of drug dose and, Delta Dose, which is the amount by which the drug dose is changed.

For treatment using a single cytotoxic drug, as per the dose modulation protocol, all delta dose values tested here, that is, delta dose=25%, 50%, or 75% increased TTP relative to standard treatment (Fig. 2.7, Table 2.6). However, when using a single cytostatic drug, as per the dose modulation protocol, only delta dose=75% leads to increased TTP relative to standard treatment (Fig. 2.7, Table 2.6). In general, choosing a high value of delta dose improves survival outcome relative to choosing a lower value for delta dose (Fig. 2.7, Table 2.6). Thus, using delta dose=50% leads to better survival outcome than using delta dose=25% for treatment using a single cytotoxic drug, and using delta dose=75% leads to better survival outcome than using delta dose=50% when using a single cytostatic drug (Table 2.6).

Table 2.6: Effect of the delta dose parameter on determining the outcome of dose modulation adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				
Delta Dose=25%	Standard Treatment	0.41	0.29-0.58	<0.001
Delta Dose=50%	Standard Treatment	0.26	0.16-0.40	<0.001
Delta Dose=75%	Standard Treatment	0.20	0.12-0.33	<0.001

Delta Dose=50%	Delta Dose=25%	0.43	0.26-0.71	<0.001
Dose Modulation (cytostatic)				
Delta Dose=25%	Standard Treatment	1.9	1.4-2.6	<0.001
Delta Dose=50%	Standard Treatment	1.7	1.2-2.4	<0.01
Delta Dose=75%	Standard Treatment	0.65	0.45-0.94	<0.05
Delta Dose=75%	Delta Dose=50%	0.54	0.35-0.83	<0.01

Effects of stopping treatment when tumor burden falls below a certain level

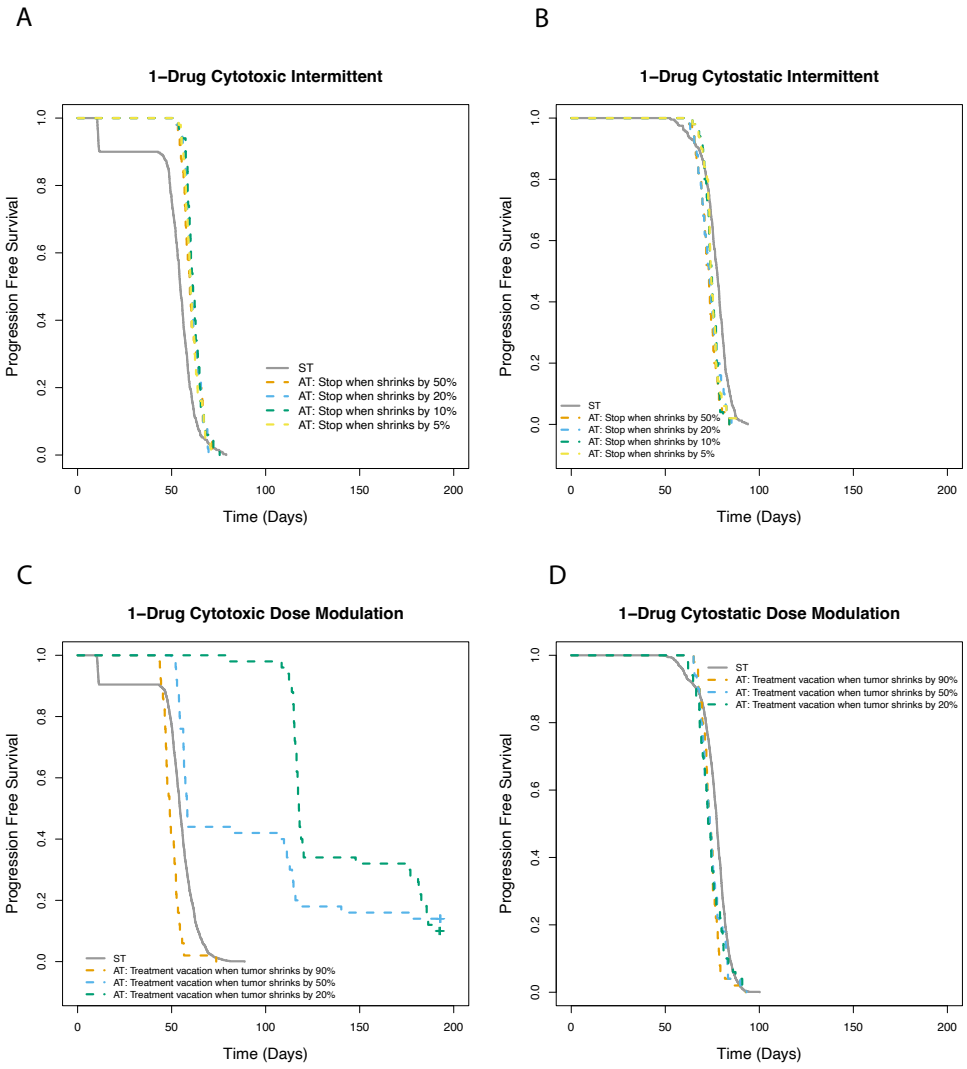

Figure 2.8: Effect of stopping treatment when tumor burden falls below a certain level for adaptive therapy using a single cytotoxic or a single cytostatic drug. For treatment as per the intermittent protocol using either a single cytotoxic (Fig. 8A), or a single cytostatic drug (Fig. 8B), the threshold for stopping treatment was varied as the tumor shrinks by 5%, 10%, 20%, or 50% of the pre-treatment baseline. Survival outcome for treatment using a single cytotoxic drug (Fig. 8C), or a single cytostatic drug (Fig. 8D) as the trigger for treatment vacation is when the tumor shrinks by 20%, 50%, or 90%.

The intermittent protocol has a key parameter: at what tumor burden should the treatment be stopped when the tumor is shrinking, in order that the tumor may be allowed to climb back up to the baseline value at which treatment was initiated previously. For

treatment using a single cytotoxic drug, as per the intermittent protocol, pausing treatment when tumor shrinks by 20%, or 50% leads to increased TTP relative to standard treatment, but no improvement in survival outcome was observed relative to standard treatment when pausing treatment when tumor shrinks by 90%. However, no improvement in survival outcome was observed for any of the values tested here when using a single cytostatic drug (Fig. 2.8, Table 2.7). In general, the sooner treatment is paused the better is the survival outcome (Table 2.7).

For dose modulation protocols, an important consideration to be made is whether treatment vacation should be triggered when tumor shrinks by at least a certain percentage relative to the start of therapy. For treatment using a single cytotoxic drug, as per the dose modulation protocol, triggering a treatment vacation when the tumor shrinks by 20% , or 50% increases TTP relative to standard treatment, but no increase in TTP relative to standard treatment is observed when a treatment vacation is triggered when the tumor shrinks by 90% (Fig. 2.8, Table 2.7). In general, the sooner a treatment vacation is triggered when the tumor is shrinking the better is the survival outcome (Table 2.7). Thus, triggering a treatment vacation when the tumor shrinks by 20% results in improved survival outcome than triggering a treatment vacation when the tumor shrinks by 50% (Table 2.7), and triggering a treatment vacation when the tumor shrinks by 50% leads to a better survival outcome than triggering a treatment vacation when the tumor shrinks by 90% (Table 2.7). When using a single cytostatic drug, there was no improvement in survival outcome for any of these values tested (Fig. 2.8D).

Table 2.7: Effect of stopping treatment when tumor burden falls below a certain level for adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	Comparison Condition	Hazard Ratio	95% CI	*p*-value
Dose Modulation (cytotoxic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	0.003	0.0004-0.0191	<0.001
Treatment vacation when tumor shrinks by 50%	Standard Treatment	0.20	0.13-0.30	<0.001
Treatment vacation when tumor shrinks by 90%	Standard treatment	2.5	1.9-3.4	<0.001
Treatment vacation when tumor shrinks by 20%	Treatment vacation when tumor shrinks by 50%	0.50	0.33-0.77	<0.01
Treatment vacation when tumor shrinks by 50%	Treatment vacation when tumor shrinks by 90%	0.12	0.07-0.20	<0.001
Intermittent (cytotoxic)				
Stop when shrinks by 5%	Standard Treatment	0.54	0.40-0.73	<0.001
Stop when shrinks by 10%	Standard Treatment	0.47	0.35-0.64	<0.001
Stop when shrinks by 20%	Standard Treatment	0.51	0.38-0.69	<0.001

Stop when shrinks by 50%	Standard Treatment	0.55	0.41-0.73	<0.001
Dose Modulation (cytostatic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	1.4	1.1-1.9	<0.05
Treatment vacation when tumor shrinks by 50%	Standard Treatment	1.4	1.0-1.8	<0.05
Treatment vacation when tumor shrinks by 90%	Standard treatment	1.8	1.4-2.4	<0.001
Intermittent (cytostatic)				
Stop when shrinks by 5%	Standard Treatment	1.8	1.3-2.4	<0.001
Stop when shrinks by 10%	Standard Treatment	2.1	1.5-2.8	<0.001
Stop when shrinks by 20%	Standard Treatment	1.9	1.4-2.6	<0.001
Stop when shrinks by 50%	Standard Treatment	2.5	1.8-3.4	<0.001

Drug dosage level at which adaptive therapy is initiated and capped

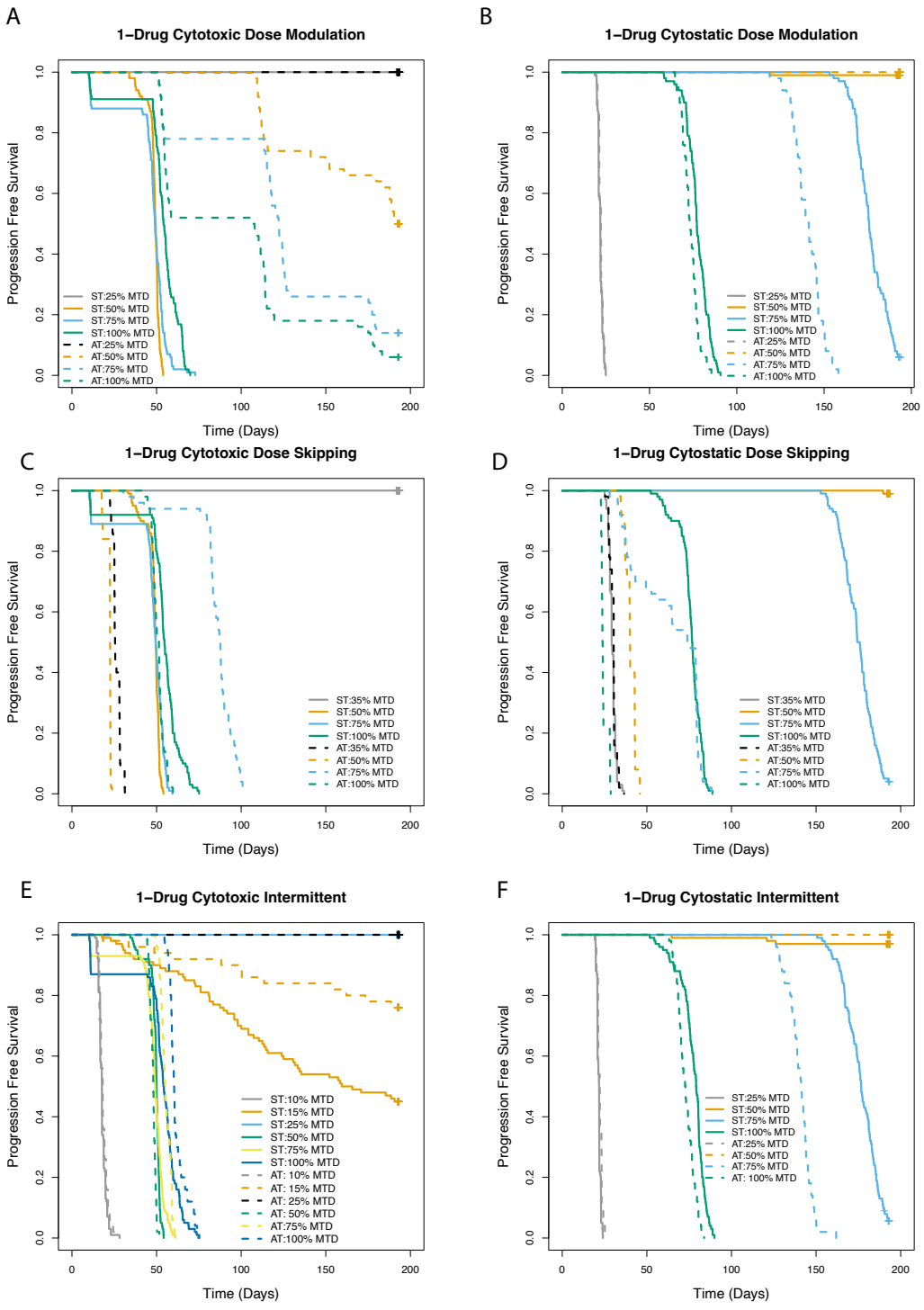

Figure 2.9: Effect of administering treatment at a range of different drug dosages for adaptive therapy using a single cytotoxic or a single cytostatic drug. Survival outcome comparing treatment as per the dose modulation protocol relative to ST, starting and capping dosing at 25%, 50%, 75%, or 100% of MTD, for treatment using a single

cytotoxic drug (Fig. 2.9A), or a single cytostatic drug (Fig. 2.9B). Survival outcome for treatment as per dose-skipping protocol administered at 35%, 50%, 75%, or 100% of MTD relative to standard treatment using either a single cytotoxic drug (Fig. 2.9C), or a single cytostatic drug (Fig. 2.9D). Survival outcome for treatment as per the intermittent protocol administered at 10%, 15%, 25%, 50%, 75%, or 100% of MTD relative to ST for treatment using a single cytotoxic (Fig. 2.9E), or a single cytostatic (Fig. 2.9F) drug.

For the dose modulation protocol, we tested different drug dosage levels for initiating treatment. We also capped the dose level at that value, so that dose modulation was never allowed to exceed that level. We observe that for treatment using a single cytotoxic drug, as per the dose modulation protocol, initiating treatment at 50% , 75%, or 100% of MTD resulted in an increase in TTP relative to standard treatment (Fig. 2.9A, Table 2.8), whereas for treatment initiation at 25% of MTD, we observed no cases of death for either the standard treatment or treatment as per the dose modulation protocol (Fig. 2.9A), and thus there was no increase in TTP with the dose modulation protocol relative to standard treatment. For treatment using a single cytostatic drug, as per the dose modulation protocol, none of the values tested, that is, 25%, 50%, 75%, or 100% of MTD resulted in increase in TTP relative to standard treatment (Fig. 2.9B).

For the dose-skipping protocol, we tested different drug dosage levels for administering the drugs, that is, 35%, 50%, 75%, or 100% of MTD. We observed an increase in TTP relative to standard treatment only for drug dosage level at 75% of MTD, but no increase in TTP relative to standard treatment for the other drug dosage levels tested here, that is 35%, 50%, or 100% (Fig. 2.9C, Table 2.8). For treatment using a single cytostatic drug, none of the drug dosage levels tested resulted in increase in TTP relative to standard treatment (Fig. 2.9D).

For the intermittent protocol, we tested different drug dosage levels for administering the drugs, that is, 10%, 15%, 25%, 50%, 75%, or 100% of MTD for cytotoxic drugs and 25%, 50%, 75%, or 100% of MTD for cytostatic drugs. We observed an increase in TTP relative to standard treatment only for drug dosage levels of 15%, 75%, and 100% of MTD (Fig. 2.9E, Table 2.8). For treatment using a single cytostatic drug, as per the intermittent protocol, only treatment with a drug dosage level at 50% of MTD resulted in an increase in TTP relative to standard treatment (Fig. 2.9F, Table 2.8).

Table 2.8: Effect of administering treatment at a range of different drug dosages for adaptive therapy using a single cytotoxic or a single cytostatic drug

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
Dose Modulation (cytotoxic)				
25% MTD	Standard Treatment at 25% MTD			Not Significant
50% MTD	Standard Treatment at 50% MTD	~0		
75% MTD	Standard Treatment at 75% MTD	0.06	0.03-0.12	<0.001
100% MTD	Standard treatment at 100% MTD	0.23	0.15-0.37	<0.001
Dose Skipping (cytotoxic)				
Standard Treatment at 35% MTD	35% MTD	~0		

Standard Treatment at 50% MTD	50% MTD	~0		
75% MTD	Standard Treatment at 75% MTD	0.014	0.004-0.048	<0.001
100% MTD	Standard treatment at 100% MTD	2.9	2.0-4.3	<0.001
Intermittent (cytotoxic)				
10% MTD	Standard Treatment at 10% MTD			Not Significant
15% MTD	Standard Treatment at 15% MTD	0.34	0.18-0.64	<0.001
25% MTD	Standard Treatment at 25% MTD			Not Significant
50% MTD	Standard treatment at 50% MTD	3.3	2.3-4.9	<0.001
75% MTD	Standard treatment at 75% MTD	0.33	0.23-0.47	<0.001
100% MTD	Standard treatment at 100% MTD	0.48	0.34-0.68	<0.001
Dose Modulation (cytostatic)				
25% MTD	Standard Treatment at 25% MTD			Not Significant

50% MTD	Standard Treatment at 50% MTD			Not Significant
75% MTD	Standard Treatment at 75% MTD	248.8	56.4-1096	<0.001
100% MTD	Standard treatment at 100% MTD	2.4	1.7-3.4	<0.001
Dose Skipping (cytostatic)				
35% MTD	Standard Treatment at 35% MTD			Not Significant
Standard Treatment at 50% MTD	50% MTD	~0		
Standard Treatment at 75% MTD	75% MTD	~0		
Standard treatment at 100% MTD	100% MTD	~0		
Intermittent (cytostatic)				
25% MTD	Standard Treatment at 25% MTD	0.53	0.37-0.77	<0.001
50% MTD	Standard treatment at 50% MTD	~0		
75% MTD	Standard treatment at 75% MTD	86.3	36.5-204	<0.001

100% MTD	Standard treatment at 100% MTD	2.7	1.9-3.9	<0.001

Median TTP versus Average Drug Dose

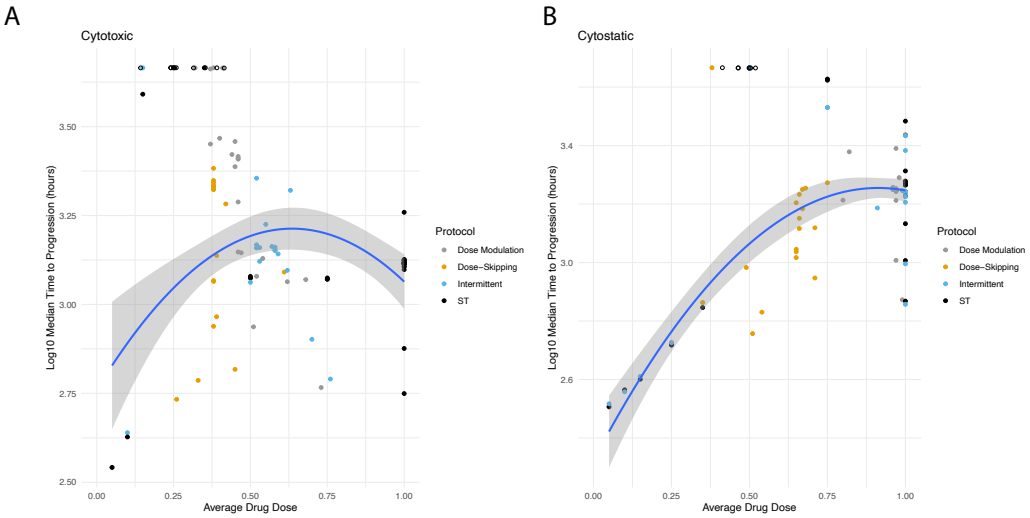

Figure 2.10: Summarizing the relationship between drug dose and time to progression for adaptive therapy using a single cytotoxic or a single cytostatic drug. In each panel, the average amount of drug used per timestep between the start of therapy and the time of progression is plotted on the X-axis, and the median time to progression for that protocol under those parameter values are plotted on the Y-axis, for treatment using either a single cytotoxic drug (A) or a single cytostatic drug (B). The points are colored based on the specific protocol. Open circles indicate data points that are censored as less than 50% of test-subjects have progressed, and are not included in the calculation. A quadratic fit to the curve along with the confidence intervals have been indicated in the figure panels. Each point represents a specific Kaplan-Meier survival curve for a given set of parameter values.

We plotted log10 median TTP versus average drug dose including all data points except for which a median TTP is not available (since less than 50% of the test subjects has progressed) and fitted the curve to a quadratic plot. For treatment using a single cytotoxic drug (Fig. 2.10A), median R-squared value is 0.1593 and adjusted R-squared is

0.1398. For treatment using a single cytostatic drug (Fig. 2.10B), median R-squared value is 0.6667 and adjusted R-squared value is 0.6592.

Discussion

Design of adaptive therapy protocols for cancer treatment is challenging as, unlike standard treatment at maximum tolerated dose, where the same drug dosage is administered every treatment cycle, adaptive therapy treatment protocols typically involve multiple parameters, in order to account for the change in tumor burden and drug levels for every treatment cycle. Further, different drugs could differ in their mode of action, which could affect treatment outcome in important ways. Most of the literature involving mathematical modeling or agent-based simulations implicitly assume a cytotoxic mode of action. In this article, we sought out to investigate three different adaptive therapy protocols, namely, dose modulation, dose-skipping, and intermittent, and standard treatment at maximum tolerated dose, using either a single cytotoxic, or a single cytostatic drug, under a variety of different settings on cell kinetics, or treatment dynamics, with the goal of finding the optimum protocol for each setting, on a case-by-case basis. While cytotoxic drugs kill cells as a function of drug concentration, cytostatic drugs, as a function of drug concentration, prevents cells from dividing.

We observed all three adaptive therapy protocols tested here, that is, dose modulation, dose-skipping, and intermittent increased TTP relative to standard treatment, when using a single cytotoxic drug, but none of the protocols increased TTP relative to standard treatment when using a single cytostatic drug, under the default values of parameter settings. In line with the preclinical adaptive therapy experiments conducted in mice with breast cancer (Enriquez-Navas et al. 2016), which involved dosing with the drug

paclitaxel, we see dose modulation outperforming dose-skipping using a single cytotoxic drug under the default parameter settings (HR=0.48, CI: 0.29-0.79, p<0.01). We did not observe increase in TTP relative to standard treatment for the intermittent protocol treament using a single cytostatic drug, and thus our results do not agree with the prostate cancer clinical trials (Zhang et al. 2017) conducted using the drug abiraterone, where the experimenters observed increase in TTP relative to a contemporaneous cohort of patients using the intermittent protocol. But our parameters are not calibrated to the individual prostate cancer patients (Zhang et al. 2017; J. West et al. 2020; J. B. West et al. 2019b; Brady-Nicholls et al. 2020), and our default values of parameters may not represent the dynamics of that system. Furthermore, hormone therapy in prostate cancer appears to shrink the tumor and so may be having both a cytostatic and a cytotoxic effect.

Our results indicate fitness cost incurred by resistant cells, as manifested in longer doubling times relative to sensitive cells in the absence of the drug, is necessary, and higher fitness cost leads to improved survival outcome in most cases when using a single cytotoxic drug. However, when using a single cytostatic drug, despite a high fitness cost, none of the treatment protocols improve treatment outcome relative to standard treatment, with two exceptions, where we see dose-skipping outperforming standard treatment under 1.7-fold, or 2.5-fold fitness cost albeit with little effect size. Our results here are consistent to our findings in an earlier publication (Thomas et al. 2022), where we observed a fitness cost of resistance is required for the multi-drug adaptive therapy protocols to work significantly better than standard treatment and that higher fitness cost translated to improved survival outcome.

The replacement parameter is an indicator of cell competition, as it specifies the likelihood that a cell can replace its neighbor if there are no empty spaces in its immediate neighborhood when it tries to divide. We observe under conditions of 0% replacement, when using a single cytotoxic drug, no increase in TTP relative to the standard treatment, but we observe an increase in TTP relative to standard treatment under conditions of 50%, or 100% replacement, with the singular exception where dose-skipping works poorly under conditions of 100% replacement, the progression being driven by total tumor burden and not the resistant cells in this case. Also, our observation that increase in replacement translates to an improved survival outcome, agrees with our earlier findings (Thomas et al. 2022) that adaptive therapy survival outcomes using multiple drugs improves under conditions of higher replacement rates. When using a single cytostatic drug, however, no increase in TTP relative to the standard treatment was observed for any of the adaptive therapy protocols tested here, regardless of the level of replacement. Stem cells are able to replace each other (Vermeulen and Snippert 2014; Vermeulen et al. 2013). Cancer cell cannibalism (entosis) is a phenomenon by which one cell kills its neighboring cell (Durgan and Florey 2018; Fais and Overholtzer 2018; Hamann et al. 2017). Because cancer cell replacement leads to cell death, cell replacement can be considered to be a special kind of cell turnover, opening up spaces for the sensitive cells to proliferate at the expense of resistant cells in the absence of the drug potentially leading to improved survival outcome with cytotoxic drugs under conditions of higher replacement rates.

The turnover parameter is another indicator of cell competition, and it measures the effect of cell death and divisions with identical doubling times for each cell type across

the two scenarios, that is low, or high turnover. In stark contrast to our findings earlier when we varied the replacement or the fitness cost parameter, where treatment using a single cytostatic drug did not lead to an increase in TTP relative to standard treatment for any of the adaptive therapy protocols tested, despite increase in fitness cost, or replacement, we observe every adaptive therapy protocol was able to increase TTP relative to standard treatment under conditions of high turnover, both for treatment using a single cytotoxic, or a single cytostatic drug. We find the turnover parameter to be critical, as it serves as an important determinant to whether treatment outcome would be better with an adaptive therapy protocol versus standard treatment. As such, markers for cell turnover would be paramount to assess the potential efficacy of adaptive therapy protocols. Strobl et al. has shown that turnover amplifies the effect of fitness cost of resistance (Strobl et al. 2021, 2022) by extending TTP. In our earlier publication (Thomas et al. 2022) we found high turnover to either increase TTP relative to standard treatment for DM cocktail tandem, or insignificant with respect to low turnover for the two ping-pong protocols tried there (DM Ping-Pong on Progression, DM Ping-Pong Every Cycle). It could be that selection for the doubly resistant cells was stronger when both drugs were used simultaneously (DM Cocktail Tandem) and less when either of the drugs were used at a time, and thus the effect of turnover wasn't noticeable as it maxed out for the two ping-pong protocols explored there. Also, there were 4 different cell types in our earlier model, in contrast to the 2 cell types here.

The dose modulation protocols have two primary parameters: Delta Tumor, which is the amount the tumor burden must change in order to trigger a change of drug dose and, Delta Dose, which is the amount by which the drug dose is changed. Similar to our

findings in the 2-drug paper, we observe all of the adaptive therapy protocols work best when using a relatively low value of delta tumor versus a high value. As such, we observe the general trend that survival outcomes decrease in the following order for the delta tumor parameter: 5%, 10%, 20%, and 40%. This observation can also be extended to treatment using a single cytostatic drug, as dose modulation protocol using a single cytostatic drug improves survival outcome relative to standard treatment only when a delta dose value=5% was used, and not under delta dose=10%,20%, or 40%. For the delta dose parameter, however, we observe improved survival outcome when a higher delta dose value is used, with the best survival outcome at delta dose=75%, for both treatment using a single cytotoxic, or a single cytostatic drug. These results are in line with our earlier findings (Thomas et al. 2022), where we noted poor survival outcomes when using a too conservative value for the delta dose parameter. In another study (Gallaher et al. 2018), a single drug adaptive therapy regimen with the dose modulation protocol with Delta Tumor=10% and Delta Dose=50% was shown to work better than Delta Tumor=5% and Delta Dose=25%. Our modeling results suggest that dose modulation with Delta Tumor=5% and Delta Dose=75% would work best for treatment using a single cytotoxic, or a single cytostatic drug.

An open question in the field of adaptive therapy is when to withhold treatment, or in other words to back off on the drug, when it is indeed feasible to do so. For intermittent treatment protocols, a key question is at what tumor burden should the treatment be stopped when the tumor is shrinking, in order that the tumor may be allowed to climb back up to the baseline value at which treatment was initiated previously. For treatment using the dose modulation protocol, we have to decide whether to withhold treatment

when the tumor is shrinking or, to continue adjusting the drug dosages. For treatment as per the intermittent protocol, for both treatment using a single cytotoxic, or a single cytostatic drug, the tumor burden relative to the baseline at which we stop dosing has no significant effect. For treatment using the dose modulation protocol, however, we observe withholding treatment when the tumor shrinks by 20% to be far better than waiting to withhold treatment until the tumor shrinks by 50%. For treatment using a single cytostatic drug, however, these effects were not significant. These results agree with our findings (Thomas et al. 2022) earlier, where we found incorporating frequent treatment vacations works best for DM Cocktail Tandem. Interestingly, it has been shown (Thomas et al. 2022; Strobl et al. 2021) that treatment vacations would provide a benefit only under conditions of strong intra-tumoral competition. However, in that publication an ordinary differential equation was used and spatial effects were not studied.

We also explored what is an optimum drug dosage level to administer at therapy initiation (for the dose modulation protocol), or at each treatment cycle (for intermittent and dose-skipping). In general, a drug dosage level at 50%, or 75% of the MTD works well for both treatment using a single cytotoxic, or a single cytostatic drug. In addition, fixed dosing with a cytostatic drug at 50% of MTD worked almost all the time, with only a few failures. We also found a variety of protocols that were able to control the tumors indefinitely:

1. Cytotoxic dose modulation starting and capped at 25% MTD (with DeltaTumor 10%, DeltaDose 50%, and stopping treatment at 50% of the initial tumor burden)

2. Cytotoxic intermittent using a fixed dose at 25% of MTD (and stopping dosing at 50% of the initial tumor burden, and restarting when it recovers to 100% of the initial tumor burden)
3. Cytotoxic fixed dosing at 25% of MTD (regardless of how the tumor responds)
4. Cytostatic intermittent with a fixed dose at 50% of MTD
5. Cytostatic dose modulation starting and capped at 50% of MTD

To some extent, standard treatment protocols at doses less than 100% of the maximum tolerated dose (such as standard treatment at 10%, 15%, 25%, 35%, 50%, or 75% of MTD) are more along the lines of metronomic therapy albeit that the treatment frequency remains the same. The effects of cancer treatment using metronomic scheduling has been studied (Benzekry and Hahnfeldt 2013; Benzekry et al. 2015). Our observation here that standard treatment could lead to improved survival outcome provided a fraction of maximum tolerated dose is used has important implications as it suggests a more personalized patient-centered approach to treatment has the potential to work significantly better in clinical settings. Furthermore, because we did not consider toxicity in our models, the benefit observed using standard treatment at low drug dosages can be reasonably expected to have been underestimated and should work even better in clinical and experimental settings.

One general principle that emerged from these simulations is that there is a sort of Goldilocks level of drug exposure. If too much drug is used, there is strong selection for resistance, and we lose control of the tumor due to the resistant clones growing out. However, if too little drug is used, we cannot keep control of the sensitive cells and the tumor grows out of control. When we analyzed the relationship between the average

amount of drug used per unit time and the time to progression, we found a significant unimodal relationship, fitting this Goldilocks principle (G. N 1837). The R-squared values on those regressions are consistent with the fact that there are many determinants of time to progression, but the average amount of drug exposure per unit of time is clearly a significant factor. A Goldilocks level of drug dosage has been observed to be optimal in tyrosine kinase inhibitors (pazopanib, which is a VEGF receptor TKI) in a clinical setting in patients with advanced renal cell carcinoma (Rini 2018)

It is not clear if drugs that are putatively cytostatic are acting as truly cytostatic drugs. For e.g., targeted therapies that affect growth factor receptors and hormone therapies for breast and prostate cancer have been shown to actually shrink the tumor. The cell killing effect of cytostatic drugs is actually due to oncogene or hormone addiction. And thus, when you take off the drug, the cells die due to the cell killing effect. Look up some oncogene and hormone addiction papers.

Our work has several limitations. We generally don't have the technology to accurately measure total tumor burden changes of 5%. We also often do not have cost effective ways to carry out those measures frequently. However, we are essentially trying to control a complex system, and lag times between changes in the system and control responses often lead to loss of control. In the future, we will be exploring 2-drug cytostatic adaptive therapy protocols.

Conclusions

Dose modulation, dose-skipping, as well as intermittent treatment protocols work well under a wide range of parameter settings when treating using a single cytotoxic drug. In contrast, there seems to be only a handful of parameter settings that improves

survival outcome when using a single cytostatic drug. In general, adaptive therapy, using either a single cytotoxic, or a single cytostatic drug, works best under conditions of high competition among the cell types, such as higher fitness cost, high levels of replacement, or high turnover. Our results suggest assaying for the amount of turnover in the cancer would be helpful for determining the likely efficacy of adaptive therapy. In general, there seems to be an intermediate level of drug we can use, which maximizes TTP, as too little leads to progression of sensitive cells and too much leads to progression of resistant cells. In fact, we found that even a constant dosing of an intermediate drug level can provide long term control even without using adaptive therapy. Our results suggest that cancer therapy could be significantly improved by the development of sensitive and accurate measures of tumor burden, that can be used frequently to track tumor response to therapy. We should note that adaptive therapy is most appropriate when the presence or emergence of therapeutic resistance is likely and cure is unattainable. If successful, adaptive therapy holds the promise of changing cancer from an acute lethal disease into a chronic disease that does not kill us.

CHAPTER 3

IN SILICO INVESTIGATIONS OF MULTI-DRUG ADAPTIVE THERAPY PROTOCOLS

Published May 30th, 2022—Cancers; DOI: 10.3390/cancers14112699

Introduction to Previously Published Work

In this work, I have sought out to investigate adaptive therapy protocols for treating cancer using two different drugs. I have explored three different dose modulation (DM) protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, and DM Ping-Pong on Progression), and two different fixed-dose protocols (FD Dose-Skipping/Drug-Holiday, FD Intermittent), along with standard treatment (ST) at maximum tolerated dose (MTD) of the cancer drugs. Because the number of treatment parameters increase exponentially with each additional drug, one challenge to this work was to come up with adaptive therapy treatment protocols that best combine two different drugs such that the survival outcome can be improved with adaptive therapy, relative to standard treatment at MTD. I investigated how these five adaptive therapy protocols perform under a wide range of different conditions of tumor kinetics, as well as varied tumor measurement and drug dosing parameters.

I have utilized hybrid agent-based models to answer these questions, the goal of this project being both identifying the optimum adaptive therapy protocol under each of these conditions, as well as to identify the various conditions under which a single adaptive therapy protocol would work best.

All the authors in this project have taken part in the research and manuscript submission process. I have assisted in developing the models, overall development of ideas, analyzing the data, preparing the figures, writing the article.

Simple Summary

Modern "adaptive therapy" approaches to cancer therapy rely on adjusting the dose of drugs as the size of the tumor changes. They hold the promise of transforming cancer from an acute lethal disease to a chronic disease we could live with, but not die from. Previous adaptive therapy experiments have used a single drug. We set out to explore how to best combine multiple drugs in these strategies. Unfortunately, there are far too many possible ways we might combine drugs in adaptive therapies to be evaluated with clinical trials. Instead, we used computer simulations of how cancers evolve in response to therapies to identify the most promising strategies that should be tested in mouse experiments and in clinical trials in the future. These promising strategies were not specific to any particular drug or particular type of cancer, and so may have general applicability for virtually all cancers.

Abstract

The standard of care for cancer patients aims to eradicate the tumor by killing the maximum number of cancer cells using the maximum tolerated dose (MTD) of a drug. MTD causes significant toxicity and selects for resistant cells, eventually making the tumor refractory to treatment. Adaptive therapy aims to maximize time to progression (TTP), by maintaining sensitive cells to compete with resistant cells. We explored both dose modulation (DM) protocols and fixed dose (FD) interspersed with drug holiday protocols. In contrast to previous single drug protocols, we explored the determinants of

success of two-drug adaptive therapy protocols, using an agent-based model. In almost all cases, DM protocols (but not FD protocols) increased TTP relative to MTD. DM protocols worked well when there was more competition, with a higher cost of resistance, greater cell turnover, and when crowded proliferating cells could replace their neighbors. The amount that the drug dose was changed, mattered less. The more sensitive the protocol was to tumor burden changes, the better. In general, protocols that used as little drug as possible, worked best. Preclinical experiments should test these predictions, especially dose modulation protocols, with the goal of generating successful clinical trials for greater cancer control.

Keywords

adaptive therapy; cancer; drug resistance; dose modulation; evolution; agent-based model

Introduction

Historically, the standard treatment (ST) for most solid tumors has been the maximum tolerated dose (MTD) of a cancer drug (Gatenby 2009; Chabner and Roberts 2005), with the ultimate goal of eradicating the tumor. However, advanced cancers often quickly evolve drug resistance under ST protocols. Cells in a cancer are heterogeneous (Williams et al. 2016; E. M. Ross and Markowetz 2016; Ricketts and Marston Linehan 2014; Morris et al. 2016; Griffiths et al. 2021; Raatz et al. 2021; Kaznatcheev et al. 2019; Marusyk, Almendro, and Polyak 2012) and constant application of high doses of drugs eliminates drug sensitive cells while drug resistant cells survive, divide, and multiply, taking over the tumor (Worsley, Mayne, and Veale 2016; Ramos and Bentires-Alj 2015; Barrett et al. 2013). In ecology, this is called competitive release (Enriquez-Navas, Wojtkowiak, and Gatenby 2015a). The same phenomenon occurs in the evolution of

pesticide-resistant pests when treated with high doses of pesticides (Adkins and Shabbir 2014; Alto et al. 2013). However, resistance to treatment typically comes at a fitness cost (Gallaher et al. 2018). That is, resistant cells face a penalty, which can be measured in increased doubling times relative to the sensitive cells (Gallaher et al. 2018). In vitro competition experiments using a co-culture of drug sensitive MCF7 and drug-resistant MCF7Dox cell line have demonstrated that, in the absence of the drug, MCF7 cells can outcompete MCF7Dox cells within a few generations (Gallaher et al. 2018). Inspired by pest management, Gatenby and colleagues have shown that robust cancer control is possible if there is a substantial fitness cost to resistance (Gatenby, Brown, and Vincent 2009a). Adaptive therapy leverages the fitness cost of resistance, using competition with sensitive cells to keep resistant cells under control (Gatenby 2009; Enriquez-Navas, Wojtkowiak, and Gatenby 2015a; Gallaher et al. 2018; Gatenby, Brown, and Vincent 2009a; Gatenby, Silva, Gillies, and Roy Frieden 2009; Enriquez-Navas et al. 2016; Zhang et al. 2017; J. West et al. 2020; J. B. West et al. 2019a; Ibrahim-Hashim et al. 2017; Bacevic et al. 2017; Buhler et al. 2021; A. Araujo et al. 2021; Brady-Nicholls et al. 2020; J. Cunningham et al. 2020; Hansen and Read 2020a). This can result in long-term containment of the tumor, especially suitable for cases where a straightforward cure is not attainable due to the presence of drug-resistant cells at diagnosis.

Recent preclinical experimental studies in mice with ovarian and breast cancer cell lines have demonstrated the superiority of dose modulation adaptive therapy over MTD treatment in maintaining a stable tumor burden and increasing time to progression (Gatenby, Silva, Gillies, and Roy Frieden 2009; Enriquez-Navas et al. 2016). A clinical

trial of adaptive therapy for metastatic castrate-resistant prostate cancer patients has extended the median time to progression to at least 27 months compared with a contemporaneous study of prostate cancer patients having a median time to progression of about 16 months with standard of care treatment (Zhang et al. 2017). Theoretical models have been useful for exploring the dynamics of cancer and novel approaches to therapy (Traina et al. 2008; Brady and Enderling 2019; Rodrigues and de Arruda Mancera 2013; Everett, Nagy, and Kuang 2016; Jain et al. 2011; Rockne et al. 2009; Benzekry and Hahnfeldt 2013; Benzekry et al. 2015; Kaznatcheev, Scott, and Basanta 2015; Bruno et al. 2020). Computational simulations using an agent-based model provide evidence that dose modulation adaptive strategies are superior in controlling cancer compared with an MTD approach, especially when the tumor is heterogenous (Gallaher et al. 2018). Optimal control theory has also been used to develop adaptive therapy protocols (Gluzman, Scott, and Vladimirsky 2020). Mathematical models have shown that adaptive therapy can work even when there is no fitness cost of resistance under some conditions (e.g., high turnover) (Viossat and Noble 2021; Strobl et al. 2021).

Most of the empirical and theoretical studies on adaptive therapy have focused on single drugs (Gallaher et al. 2018; Gatenby, Silva, Gillies, and Roy Frieden 2009; Enriquez-Navas et al. 2016; Zhang et al. 2017; Bacevic et al. 2017), whereas most chemotherapy protocols for cancer involve multiple drugs (Delbaldo et al. 2004; Wagner et al. 2006; Carrick et al. 2009; Mokhtari et al. 2017). Can we improve on adaptive therapy protocols by using multiple drugs? Adaptive therapy protocols already involve more variables than standard, fixed dose protocols, and the addition of multiple drugs leads to a combinatorial explosion in the number of possible adaptive therapy protocols.

It is, therefore, impractical to test many different protocols in mice. Mathematical and game theory models for multi-drug adaptive therapy have previously been developed to find the best way to combine two drugs to treat metastatic castrate-resistant prostate cancer (J. West et al. 2020; J. B. West et al. 2019a; J. Cunningham et al. 2020). Mathematical control theory has also been used to formulate a multidrug regimen for leukemia (Moore 2018). However, these previous modeling efforts either do not include spatial structure (J. West et al. 2020; J. B. West et al. 2019a), which can have dramatic effects on the clonal competition in cancers (Bacevic et al. 2017), or their results depend, in part, on the cost of resistant cells allowing sensitive cells to encapsulate them and thereby prevent resistant cells from proliferating (Gallaher et al. 2018; Bacevic et al. 2017). More recent work from Strobl and colleagues (Gallaher, Brown, and Anderson 2019; Strobl et al. 2022; Bacevic et al. 2017)explicitly quantifies this spatial competition between sensitive and resistance cells.

Our main goal here was to explore how we might best combine drugs in adaptive protocols to prevent therapeutic resistance from spreading in a tumor. In addition, we wished to address some caveats of previous models and test how different types of spatial constraints affect adaptive therapy protocols. Moreover, traditional differential equation models of multi-drug adaptive therapy do not capture these spatial interactions which can, nevertheless, play an important role and affect treatment outcome in important ways. In this study, we modelled multi-drug adaptive therapy with two drugs, exploring five different adaptive therapy protocols, and the standard treatment (ST), at maximum tolerated dose until progression, using a hybrid agent-based model (Bravo et al. 2020), under different assumptions of spatial constraints on clonal competition. We investigated

protocols that either applied both drugs at the same time, or alternated them in some fashion, by either adjusting the dose or using a fixed dose with some form of drug holiday. Our primary outcome was time to progression, which was defined by the average tumor burden exceeding a threshold set by the control condition of no treatment, or the proportion of cells that were resistant to both drugs expanding to such a significant size (20% of the simulated space) that the protocol was doomed to failure. In this way, we sought to identify multi-drug adaptive protocols that could achieve long-term control of therapeutic resistance.

Materials and Methods

We described the details of our agent-based model using the standard overview design details (ODD) format from Grimm et al. (Grimm et al. 2010). We implemented our model by utilizing the Hybrid Automata Library (HAL), which is a hybrid agent-based modeling framework designed to model discrete cell agents interacting with continuous chemical dynamics (Bravo et al. 2020).

Purpose

Our goal was to determine how to combine drugs in an adaptive therapy protocol in order to increase time to progression (TTP), and hopefully prevent progression altogether.

Entities, State Variables, and Scales

We modelled a tumor as a collection of interacting cells located on a 100 by 100, 2-dimensional square lattice. Only one cell could occupy a lattice location at a time, and cells were restricted to being only on the lattice. We used this relatively small cell population size due to computational constraints. While this was smaller than is realistic

for even a small tumor (Fortunato et al. 2017), we compensated by using a high mutation rate, making the tumor far more difficult to cure with chemotherapy. For the 2-drug regimen, there were four cell types: doubly sensitive cells (sensitive to both drugs), cells resistant to drug 1, cells resistant to drug 2, or doubly resistant cells (resistant to both drugs).

Process Overview and Scheduling

At every time step, the scheduler (Fig. 3.1) updated the local drug concentration in each lattice site, iterating over each cell in a random order. Whether the cell survived or died depended on cell death probability (see *Cell Death*), which depended on both the background death probability and the probability of death due to drug treatment. If the cell survived, it might divide, subject to the division probability of the particular cell type (see *Cell Division*) and whether or not space was available in the adjacent Moore neighborhood. If space was available, the cell divided, creating a daughter cell, randomly placing it in one of the available spaces in its Moore neighborhood. However, if no space was available, the dividing cell might be able to replace a neighboring cell depending on the replacement probability (see *Competition for Space*). Daughter cells could mutate every time a cell underwent division (see *Mutation*). Doubly sensitive cells could mutate to become doubly resistant cells in one step or via an intermediate step by becoming a singly resistant cell. Cells could mutate in both forward and reverse directions (Fig. 3.2).

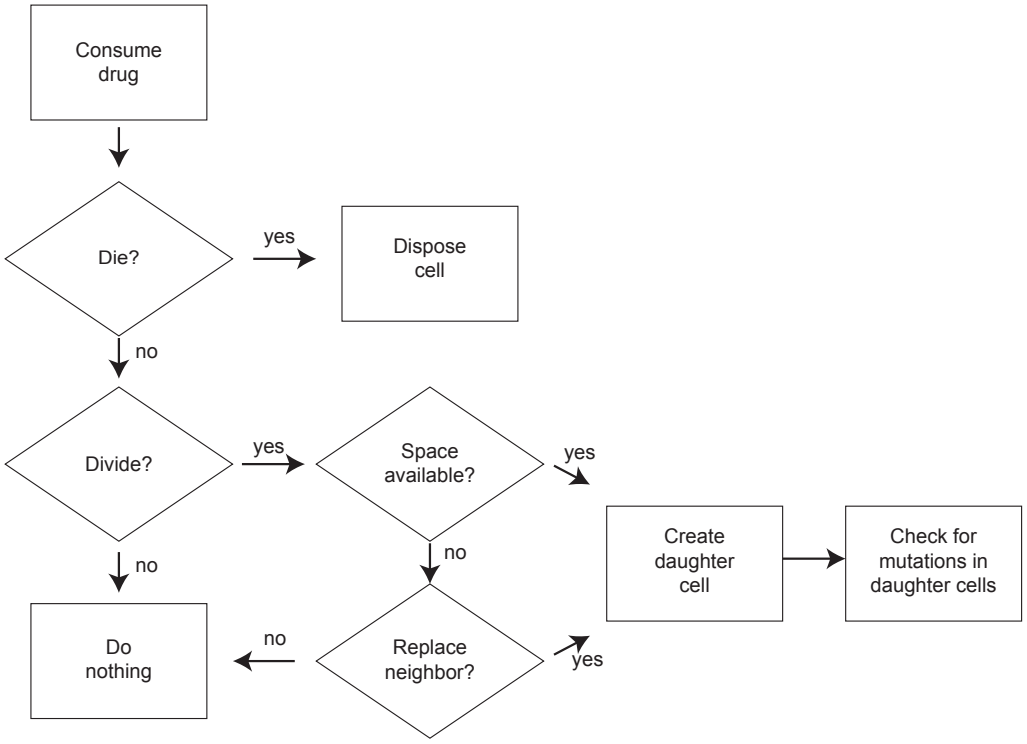

Figure 3.1: Process overview and scheduling. Cells die as a function of their sensitivity to the drugs, the available drug concentrations and the background death function. A cell divides as a function of its doubling time (resistant cells have slower doubling times). The effects of cell crowding, cell cannibalism and contact inhibition are represented by a probability of replacing a neighbor if there is no open space.

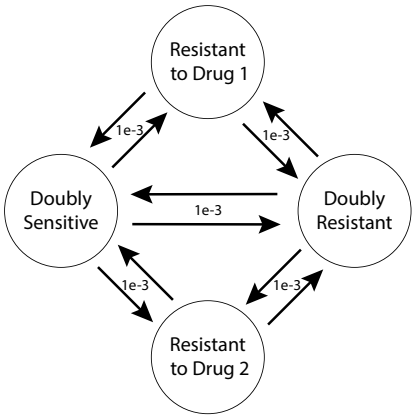

Figure 3.2: Mutation Schematic. A given cell type can mutate to any other cell type but itself with an equal probability of 10^{-3} per cell division. Doubly sensitive cells can mutate to become doubly resistant cells in one step (e.g., due to multiple drug resistance mechanisms (Benzekry et al. 2015)) or via an intermediate step of singly resistant cells. Resistant cells can also mutate to become sensitive again. This may represent epigenetic forms of resistance that are easily reversible.

Design Concepts

Basic Principles

The basic principles are: (1) tumors are heterogeneous with respect to sensitivity and resistance to drugs; (2) competition between different cell types in tumors is local, between neighboring cells; and (3) drug dynamics occur over continuous space and at a faster time scale than cell division. These features of cancer therapy make a hybrid agent-based model ideal for capturing the dynamics of cancer evolution in response to therapy.

Emergence

We observed clonal dynamics and in particular, the evolution of therapeutic resistance. We routinely observed competitive release in addition to selection for different cell types.

Adaptation

Cells in the model evolved a resistance adaptation to the drugs, encoded in the four possible cell types.

Objectives

The fitness function for the agents was implicit in the model. Different cells had different division and death rates (probabilities per unit time). Natural selection, and selection due to the cancer drugs, acted upon those phenotypes.

Learning

Not applicable.

Prediction

Not applicable.

Sensing

Cells could be killed by the amount of drug in their local environment, but they did not make any decisions based on sensing that concentration.

Interaction

Cells interacted with one another directly when they divided and replaced a neighboring cell (see *Competition for Space*).

Stochasticity

Stochasticity was an important feature of our model as cell death, division, mutation and replacement are stochastic processes. For each protocol with a given set of parameter values, we ran the model at least 50 times to account for this stochasticity.

Collectives

There were no collectives in the model.

Observation

We ran the model for 5000 time steps (approximately 208 days), with each time step representing 1 h. We determined whether the tumor had "Progressed" or was "Controlled", and we also recorded the time at which the tumor "Progressed". Progression was defined by the following survival criteria: if the rolling average of tumor burden over a period of 500 time steps equaled or exceeded 98% of the carrying capacity, or if the rolling average of the total number of doubly resistant cells over a period of 500 time steps equaled or exceeded 20% of the carrying capacity, then the particular run was scored as "Progressed" and the time at which the progression took place was noted. We included the percent of resistant cells in our progression criteria because they were the clinically important population that could not be controlled medically and if they became common would eventually lead to an uncontrollable tumor. The 20% threshold for the doubly resistant cells was a somewhat arbitrary choice to identify cases where the doubly resistant population had started to grow out of control. However, we never observed cases where that doubly resistant population could be controlled above that threshold. Every run was replicated 50 times with a seed that was based on the clock, and we generated a summary of those runs with Kaplan–Meier curves, and analyzed the results with Cox proportional hazard regressions, which calculated the hazard ratio (HR). The

HR was the chance that the tumor would meet the progression criteria in the next time interval in the experimental group (e.g., an adaptive therapy protocol) compared with some control group (usually the standard treatment protocol).

Initialization

Parameters were set at the start of a model run depending on user input or an input file (default values are shown in Table 3.1). Tumor cells were seeded, approximately at the center of the grid, in the form of a disc of radius 10 units, containing about 400 cells, such that each cell had an equal probability of being assigned to be one of the four cell types (see *Entities, State Variables, and Scales*). We seeded the random number generator from the clock for every run of the model.

Table 3.1: Parameter table with default values.

Parameter	**Value**
Cell division rate: doubly sensitive	0.06 per hour
Cell division rate: resistant to drug 1	0.04 per hour
Cell division rate: resistant to drug 2	0.04 per hour
Cell division rate: doubly resistant	0.02 per hour
Background death rate	0.01 per hour
Replacement probability	0.5
Delta Tumor	10%
Delta Dose	50%
Probability of death due to drug 1 potency (Ψ1)	0.04 per unit drug concentration
Probability of death due to drug 2 potency (Ψ2)	0.04 per unit drug concentration
Maximum tolerated dose (MTD)	5.0 units for a single drug. See Section 2.7.6 for MTD under combination therapies.
Minimum drug dose	0.5 units
Drug on time	1 h
Frequency of drug application	Once every 24 h
Check tumor burden	Every 3 days
Drug decay	10% per hour
Drug diffusion rate	2.0

Tumor size triggering treatment	Tumor burden is 50% or more of the carrying capacity
Mutation rate	10−3 per cell division
Measurement noise standard deviation (SD)	5 cells
Total grid size	100 by 100
Duration of simulation	5000 h
Stop dosing/initiate treatment vacation when (DM protocols only):	Tumor burden is less than or equal to 25% of carrying capacity

Input Data

Not applicable.

Submodels

Cell Death

Probability of cell death in a time step (1 h) was the sum of the background death probability and probability of death due to individual drugs. Every cell type had the same intrinsic background death rate set at 0.01 per hour, for the default parameters. The equation of probability of cell death for a 2-drug regimen was as follows: probability of cell death per hour = background death probability per hour + S1*[Drug1]*Ψ1 + S2*[Drug2]*Ψ2, where S1 and S2 are binary indicator variables for the cell's sensitivity to drugs 1 and 2, respectively, such that a value of 1 indicates sensitivity, and a value of 0 indicates resistance to the particular drug; [Drug1] and [Drug2] are the concentration of those drugs (non-negative real values); and Ψ1 and Ψ2 are the drug potency (non-negative real values) of the corresponding drug, quantified as the probability of cell death per unit drug concentration per hour. The probability of cell death never exceeded 1 in any of our parameter settings.

Cell Division

As long as a cell did not just die, it had a chance to divide each time step, determined by the cell division rate of the particular cell type (Table 3.1). To incorporate the fitness cost of resistance in our model, division probabilities for the cell types were arranged in decreasing order, as follows: doubly sensitive cells > resistant to drug 1 = resistant to drug 2 > doubly resistant cells. Thus, the doubly sensitive cells had the highest, singly resistant cells to either drugs had intermediate, and doubly resistant cells had the lowest cell division rates.

Competition for Space

If there were no empty spaces adjacent to a dividing cell, it might replace one of its neighbors, with a probability set by the replacement probability parameter. This was a computationally efficient abstraction to deal with a gap in the scientific literature. We do not know the relationship between cell crowding and cell death. Some level of cell crowding must kill cells. There is evidence that crowding can collapse local capillaries (R. P. Araujo and McElwain 2004; Boucher and Jain 1992). Modeling capillary dynamics or elastic tissues, where cells could push aside neighbors, is computationally expensive. We do know it is common that cancer cells consume their neighbors (Durgan and Florey 2018; Fais and Overholtzer 2018). Contact inhibition is also a common phenomenon where the presence of neighbors inhibits cell division (Ribatti 2017; Mendonsa, Na, and Gumbiner 2018). Setting different values of the probability (r) for replacing a neighbor can represent a spectrum of behavior, from contact inhibition ($r = 0$) to neighbor killing ($r = 1$), with neighbor death due to crowding in the middle.

Mutation

The possible transitions between cell types are shown in Fig. 3.2. These transitions represent mutations that occur at a constant rate determined by the mutation rate parameter (the default is 10^{-3} per cell division, Table 3.1). We chose this particular value so as to make the effective mutation rate comparable to real tumors (Fortunato et al. 2017), where tumor burden commonly approaches a billion or more cells. Moreover, a high mutation rate ensured that doubly resistant cells were constantly being generated during our simulations, thus limiting treatment protocols that worked just because the doubly resistant cell population was small. The high mutation rate and the ability to interconvert between the cell types may also represent epigenetic mechanisms of therapeutic resistance (Brown et al. 2014; Garcia-Martinez et al. 2021).

Drug Dynamics (Diffusion and Metabolism)

Drugs were applied once a day (the frequency of drug application parameter) for one hour (drug on time parameter; Table 3.1). We made drug delivery uniform throughout the lattice such that each lattice site received the same amount of drug, representing a well perfused tumor. A fraction (10%, Table 3.1) of each drug decayed at every time-step. Cells were exposed to the remaining drug which could also freely diffuse into neighboring lattice sites. Drug diffusion was modeled using the alternating direction implicit (ADI) method (Bravo et al. 2020).

Drug Protocols

For all adaptive therapy protocols explored here, dose modulation (DM) and fixed dose (FD), tumor burden was monitored every 3 days. We modeled error in the tumor burden measurement by adding noise (drawn from a Gaussian distribution with a mean of the current tumor burden and a standard deviation of 5 cells). We initiated treatment at

MTD as soon as the tumor burden equaled or exceeded 50% of carrying capacity (5000 cells).

For the DM protocols, starting with MTD, dosage of the drugs was increased by the Delta Dose parameter if the tumor grew above the Delta Tumor threshold, and the dosages were decreased by the Delta Dose parameter if the tumor shrank by at least the Delta Tumor threshold. If the tumor grew by no more than the Delta Tumor threshold or shrank less than the Delta Tumor threshold, then the same drug dosage was administered in the next cycle. We also took the absolute tumor burden into consideration (called maximum tolerable tumor burden). If the current tumor burden exceeded the maximum that had been recorded so far, then the dosage was increased by Delta Dose. This occurred so as to prevent the tumor from growing progressively below our 'Delta Tumor' threshold for consecutive treatment cycles. In addition, if the tumor burden ever fell to, or below, the "stop dosing" threshold (which defaulted to 2500 cells), a treatment vacation was triggered, during which no drug was administered for the treatment cycle. This modelled the common clinical practice of stopping treatment if the tumor burden falls below detectable levels. Finally, we assumed that it may be difficult to formulate a cancer drug at very low dosages, so we set a minimum drug dose. If a DM protocol would cause the dose to fall below that level, we kept the dose at the minimum dose.

For the FD protocols, drug dosage was set at 75% of the MTD with a cocktail formulation, to match the experiments in (Enriquez-Navas et al. 2016). FD Intermittent relied solely on the value of the absolute tumor burden.

MTD for either drug administered singly was 5 units. We assumed that due to increased toxicity of combining drugs, the maximum that could be applied was 3 units of each drug

when they were used in combination. For fixed dose adaptive therapy protocols, we used a drug cocktail that was 75% of the MTD for each drug (0.75 of 3 units of each drug, which was 2.25 units of each drug (Enriquez-Navas et al. 2016)), however we did not observe a difference between 75% and MTD for fixed dose protocols. We investigated five different multi-drug adaptive therapy protocols and compared them to a standard treatment as follows (Fig. 3.3).

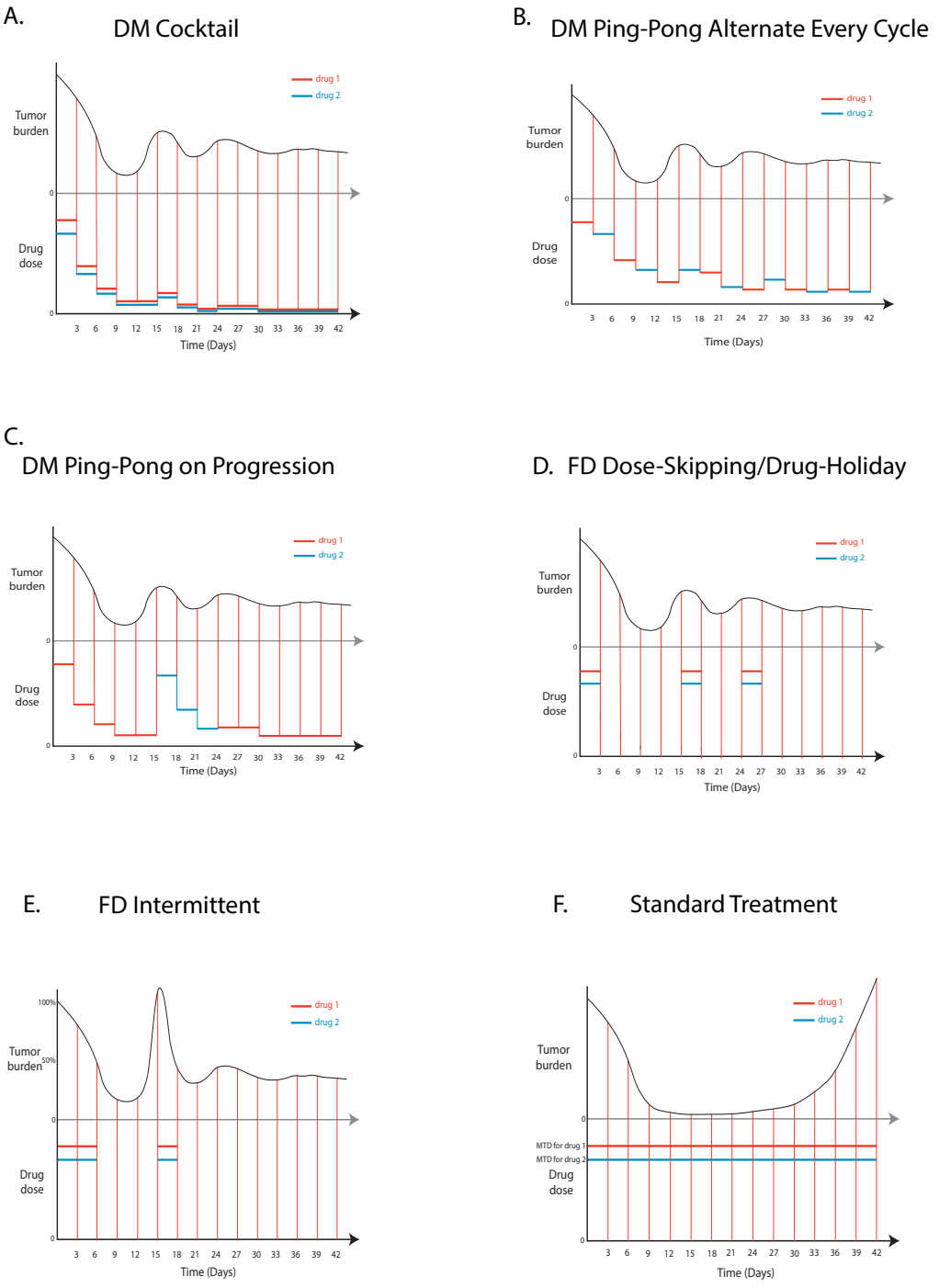

Figure 3.3: Two-drug adaptive therapy protocols, comparing variations of dose modulation (DM) and fixed dose (FD) adaptive therapy. Each panel shows on top, an example of how tumor burden might fluctuate over time, and below, how the dosing of the two drugs would be adjusted in response to the change in the tumor burden. Tumor

burden is measured every 3 days (indicated with vertical lines). (**A**) DM Cocktail increases the dose of both drugs if the tumor is growing, and reduces the dose of both drugs if the tumor is shrinking. (**B**) DM Ping-Pong Alternate Every Cycle uses one drug at a time, but alternates drugs every 3 days, and adjusts the dose depending on how the tumor responded to the drug the last time it was applied. (**C**) DM Ping-Pong on Progression also uses one drug at a time, reducing the dose if the tumor is shrinking, but switching drugs if the tumor grows. (**D**) FD Dose-Skipping/Drug Holiday is similar to the AT-2 algorithm from (Gallaher et al. 2018), a fixed dose is applied every time the tumor grows, but the dose is skipped if the tumor remains stable or shrinks. (**E**) FD Intermittent is similar to the adaptive therapy prostate cancer trial from (Gatenby, Brown, and Vincent 2009b), where a fixed dose is applied until the tumor shrinks below 50% of its initial size. Dosing is restarted if the tumor grows above 100% of its original size. Tick marks on the tumor burden axis at 50% and 100% represent absolute values that trigger administering or withholding dosages of drugs for FD Intermittent. (**F**) Standard treatment applies both drugs at maximum tolerated dose (MTD).

Standard Treatment (ST)

Both drugs (drug 1 and drug 2) were administered at maximum tolerated dose (MTD) in a cocktail formulation once every 24 h for the entire duration of the simulation (Fig. 3.3F).

DM Cocktail Tandem

Treatment started at MTD for both drugs, and dosages of both drugs were adjusted simultaneously according to the dose modulation adaptive therapy protocol, parameterized by Delta Tumor and Delta Dose (Fig. 3.3A). This was equivalent to the standard dose modulation adaptive therapy protocol (AT-1) from previous experiments (Gatenby, Silva, Gillies, and Roy Frieden 2009; Enriquez-Navas et al. 2016), but using two drugs in tandem, as if they were one.

DM Ping-Pong Alternate Every Cycle

Treatment started with drug 1 at MTD followed by drug 2 at MTD during the subsequent cycle. Drugs were always switched every cycle and dosages of each drug

were adjusted (Delta Dose) based on the response of the tumor (Delta Tumor) the last time the same drug was administered (Fig. 3.3).

DM Ping-Pong on Progression

As in the standard dose modulation protocol with a single drug, we decreased the dose by Delta Dose when the tumor shrank by at least Delta Tumor. However, if the tumor grew by more than Delta Tumor, instead of increasing the dose of the current drug, we switched to the other drug. Any time we resumed the use of a drug that was used previously, we restarted treatment with a dose that was Delta Dose higher than the last time that drug was used (because the tumor grew the last time that drug was used at the prior concentration). Initially, each drug was started at MTD. Therefore, as long as the tumor was stable or shrinking, we continued using the current drug (Fig. 3.3C).

FD Dose-Skipping/Drug Holiday

Drugs were administered in a cocktail formulation at a fixed dose that was set at 75% of the MTD. If the tumor grew by more than Delta Tumor since its last measurement, or if the tumor burden exceeded its previous maximum size, the drug was applied. Otherwise, the dose was skipped (Fig. 3.3D). This was the AT-2 protocol from (Enriquez-Navas et al. 2016), except we used two drugs in combination.

FD Intermittent

Treatment started at 75% of the MTD using a cocktail formulation. Drug was administered once every 24 h. Treatment stopped any time the tumor burden fell by at least 50% of the value at which treatment was initiated. Treatment restarted if the tumor burden ever grew by at least 100% of the value at which treatment was initiated (Fig. 3.3E). This was the protocol that was used for abiraterone in the prostate cancer clinical

trial (Zhang et al. 2017), except that we started and stopped two drugs in combination, rather than one.

Results

Dose Modulation Adaptive Therapy Protocols with Two Drugs Leads to Increased Time to Progression (TTP) Relative to Standard Treatment (ST) at Maximum Tolerated Dose

Relative to standard of care treatment (ST) at maximum tolerated dose, we observed improved TTP with DM protocols (DM Cocktail: HR = 0.25 [0.18–0.35], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.26 [0.18–0.38], $p < 0.001$; and DM Ping-Pong on Progression: HR = 0.13 [0.08–0.22], $p < 0.001$), but not the FD protocols, which actually performed worse than ST (FD Intermittent: HR = 1.67 [1.25–2.24], $p < 0.001$; FD Dose-Skipping/Drug Holiday: HR = 1.65 [1.23–2.21], $p < 0.001$), (Fig. 3.4A and Appendix B: **Supplementary Table S1**). The average total amount of drug used in the adaptive therapy protocols was less than the standard treatment (DM Cocktail: 52.1% (drug 1) and 52.1% (drug 2); DM Ping-Pong Alternate: 32.6% (drug 1) and 66.5% (drug 2); DM Ping-Pong on Progression: 34.0% (drug1) and 39.8% (drug 2); FD Intermittent: 66.2% (drug 1) and 66.2% (drug 2); and FD Dose-Skipping/Drug Holiday: 35.9% (drug 1) and 35.9% (drug 2) of ST). Fig. 3.4B–F shows the population dynamics of the different cell types over time for example runs. The number of doubly resistant cells (shown in red) progressively increased for ST (Fig. 3.4B). FD Dose-Skipping/Drug Holiday and FD Intermittent (Fig. 3.4E,F) also led to treatment failure. For treatment with DM Ping-Pong on Progression, Fig. 3.4C shows an example run in which the number of doubly resistant cells were kept in check, while Fig. 3.4D depicts the less

common case where dose modulation failed. Preventing reverse mutations, which may better model genetic resistance mutations, did not significantly change the results (Appendix B: **Supplemental Figure S2 and Table S8**).

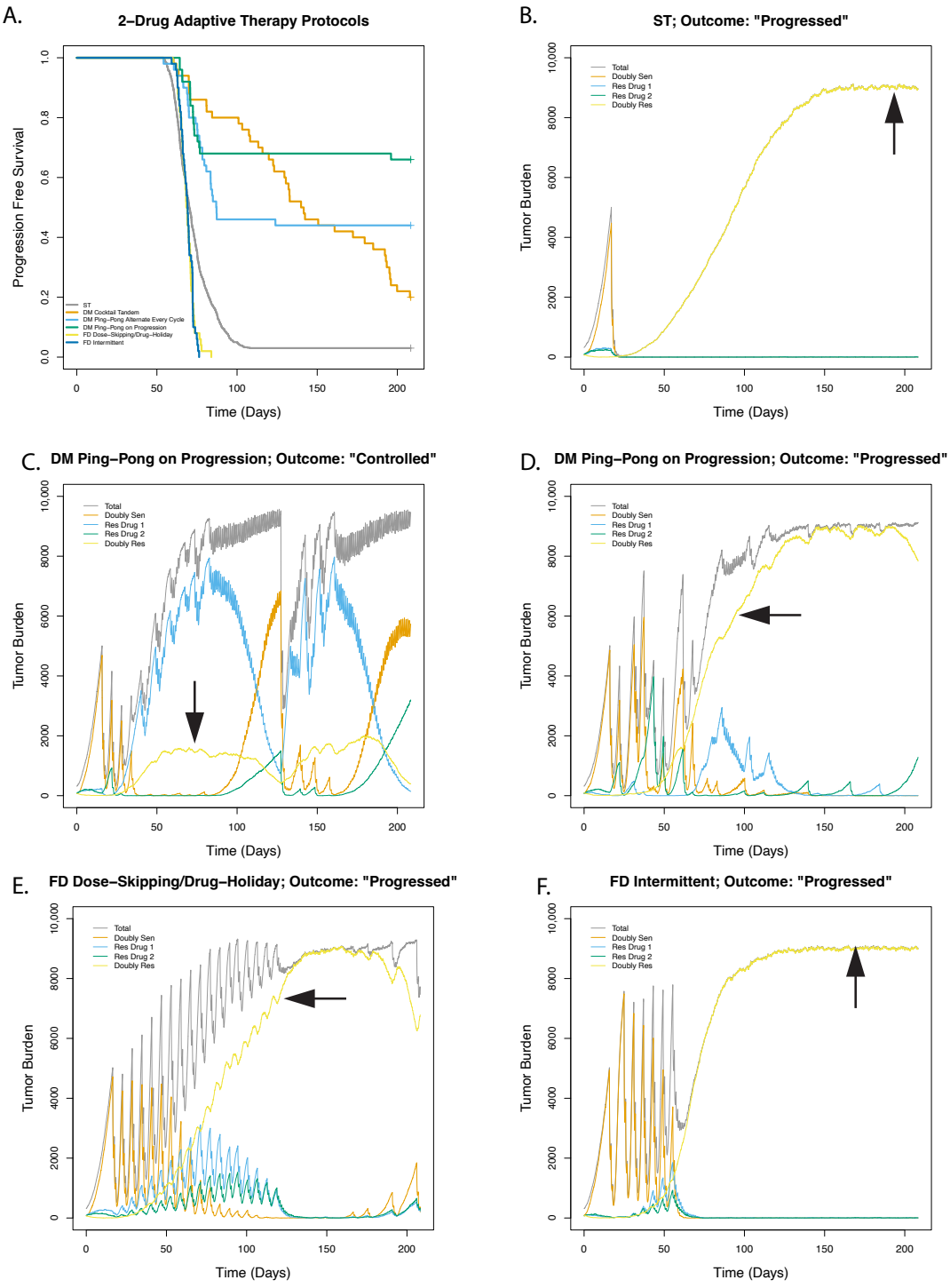

Figure 3.4: Two-drug therapies, comparing standard of care standard treatment (ST) versus variations of dose modulation (DM) and fixed dose (FD) adaptive therapy. Tumor burden was measured every 3 days. ST applied the maximum tolerated dose at each measurement. (**A**) Survival curves for DM adaptive therapy protocols (DM Cocktail

Tandem, DM Ping-Pong Alternate Every Cycle, and DM Ping-Pong on Progression) and FD adaptive therapy protocols (FD Dose-Skipping/Drug Holiday and FD Intermittent) compared with ST. The dose modulation protocols uniformly worked better than the other protocols. (**B**) Cell population dynamics for a tumor treated with the ST protocol (continuous MTD). Therapy started at about day 20 when the tumor reached 5000 cells. The results clearly show the effects of competitive release, leading to rapid progression. (**C**) Cell population dynamics for a tumor treated with the DM Ping-Pong on Progression protocol, controlling the doubly resistant cells. There was a dip in tumor burden at about 125 days owing to switching from a low dose of drug 1 to a high dose of drug 2. (**D**) Cell population dynamics for a tumor treated with the DM Ping-Pong on Progression protocol resulting in the less frequent outcome of progression. At around day 70, therapy killed almost all the sensitive and singly resistant cells, leaving insufficient cells to keep the doubly resistant cells in check. (**E**) Cell population dynamics for a tumor treated with FD Dose-Skipping/Drug Holiday resulting in rapid progression. (**F**) Cell population dynamics for a tumor treated with the FD Intermittent protocol resulting in progression. Populations of the doubly resistant cells (in yellow) are indicated by arrows. In addition, the total tumor burden (gray), the number of cells sensitive to both drugs (Doubly Sen, in orange), the number of cells resistant to drug 1 but sensitive to drug 2 (Res Drug 1, in sky blue), and the number of cells resistant to drug 2 but sensitive to drug 1 (Res Drug 2, in bluish green), are shown.

Greater Fitness Costs for Resistant Cells Increases the TTP for Adaptive Therapy

We explored the impact of the fitness cost parameter on TTP for treatment with ST and the adaptive therapy protocols (Fig. 3.5 and Appendix B: **Supplementary Table S2**). Treatment with every dose modulation protocol increased TTP relative to ST (DM Cocktail: HR = 0.25 [0.18–0.35], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.26 [0.18–0.38], $p < 0.001$; and DM Ping-Pong on Progression: HR = 0.13 [0.08–0.22], $p < 0.001$) when the fitness penalty incurred by the resistant cells was 5X (meaning that the net growth rate of the doubly sensitive cells was five times that of the doubly resistant cells, and the net growth rate of the singly resistant cells was three times that of the doubly resistant cells). When the fitness penalty incurred by the doubly resistant cells was 3X (and singly resistant cells had twice the growth rate of the doubly resistant cells), treatment with the dose modulation protocols relative to ST results in TTP was either not

significantly different (DM Cocktail: not significant [$p = 0.297$]; DM Ping-Pong Alternate: not significant [$p = 0.706$]) or was worse (DM Ping-Pong on Progression: HR = 1.34 [1.00–1.79], $p = 0.0482$). In contrast, the fixed dose protocols, FD Intermittent and FD Dose-Skipping/Drug Holiday, were either worse or not significantly different than ST, regardless of whether or not the fitness cost for the resistance cells was 3X (FD Intermittent: not significant [$p = 0.344$]; FD Dose-Skipping/Drug Holiday: HR = 1.47 [1.10–1.97], $p = 0.00851$) or 5X (FD Intermittent: HR = 1.67 [1.25–2.24], $p< 0.001$; FD Dose-Skipping/Drug Holiday: HR = 1.65 [1.23–2.21], $p < 0.001$).

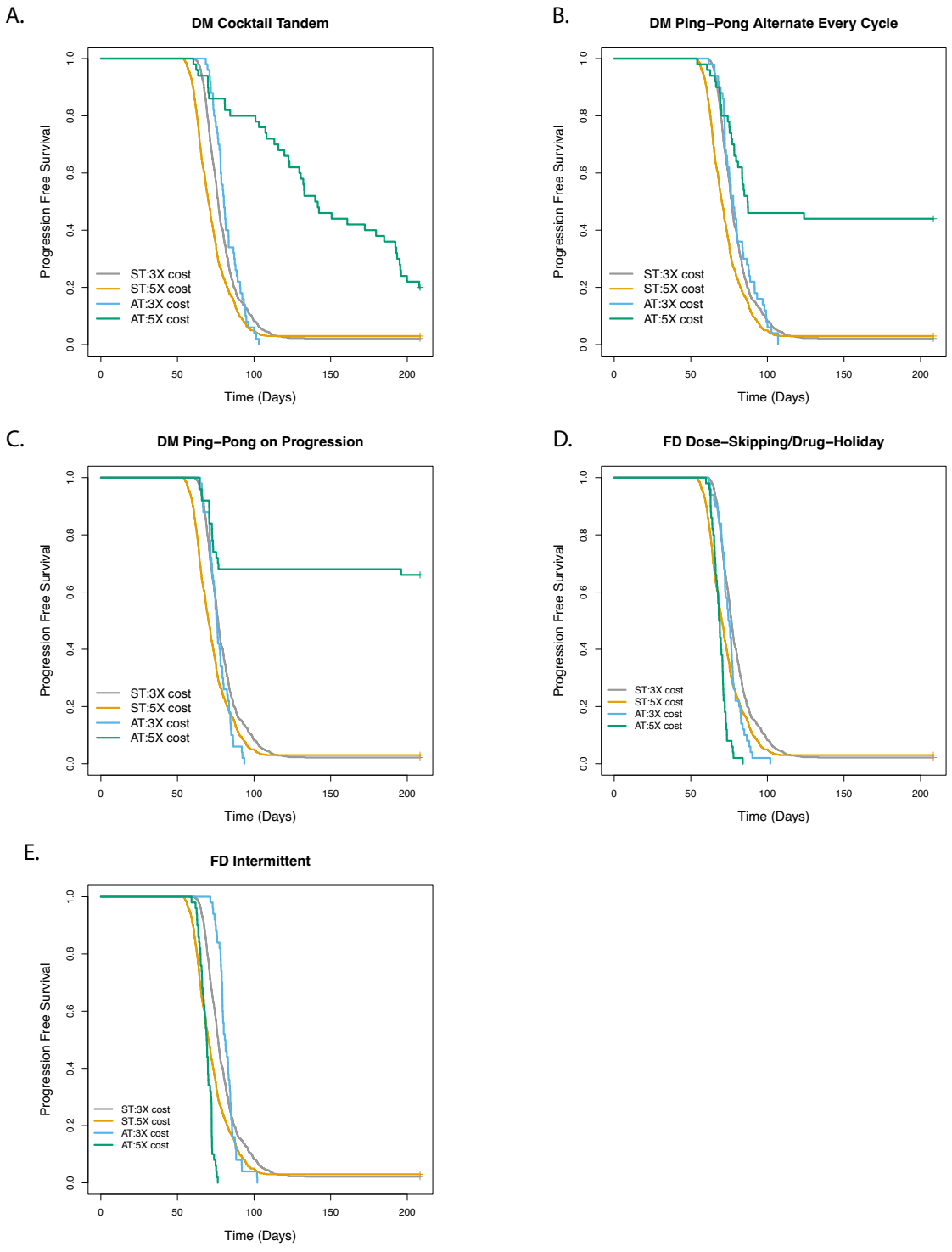

Figure 3.5: Role of fitness cost in determining the outcome of adaptive therapy with 2 drugs. The panels show the comparison of adaptive therapy (AT) versus standard treatment (ST) as fitness cost for resistance is varied for (**A**) DM Cocktail Tandem, (**B**) DM Ping-Pong Alternate Every Cycle, (**C**) DM Ping-Pong on Progression, (**D**) FD Dose-

Skipping/Drug-Holiday, (**E**) FD Intermittent. 5× fitness cost is the default, with the division rate of doubly sensitive cells being 0.06/h, doubly resistant cells being 0.02/h, and that of the singly resistant cells being 0.04/h, while the death rate of all cell types was 0.01/h, translating to a net growth rate of the doubly sensitive cells at 5 times (5×) that of the doubly resistant cells. We compared this to a 3× fitness cost, with the division rate of doubly sensitive cells being 0.04/h, doubly resistant cells being 0.02/h, and singly resistant cells being 0.03/h, while the death rate of all cell types was 0.01/h, translating to a net growth rate of the doubly sensitive cells at 3 times (3×) that of the doubly resistant cells.

Higher Levels of Cell Turnover Increases the Efficacy of Adaptive Therapy

For any given net growth rate, there can be more or less cell turnover generating that rate (determined by the cell death and division probabilities). We explored how the degree of cell turnover impacted adaptive therapy protocols (Fig. 3.6). We kept the net growth rates of the different cell types the same as our default parameters (Table 3.2), but defined a low turnover condition with a cell death rate of 0.005/h for all cell types (half of the default value of 0.01/h) and division rates of doubly sensitive cells at 0.055/h, singly resistant cells at 0.035/h, and doubly resistant cells at 0.015/h. The high turnover condition had a death rate of 0.02/h for all cell types (twice the default), division rates of 0.07/h for the doubly sensitive cells, 0.05/h for the singly resistant cells, and 0.03/h for the doubly resistant cells. The doubling times in Table 3.2 were within the range of observed human cell division times, namely, from lymphocytes that can divide in less than 10 h (Maur, Auf der Maur, and Berlincourt-Böhni 1979; Dowling et al. 2014; Yoon, Kim, and Braciale 2010), to common cancer cell lines in culture that range from 17 to 80 h doubling times (D. T. Ross et al. 2000).

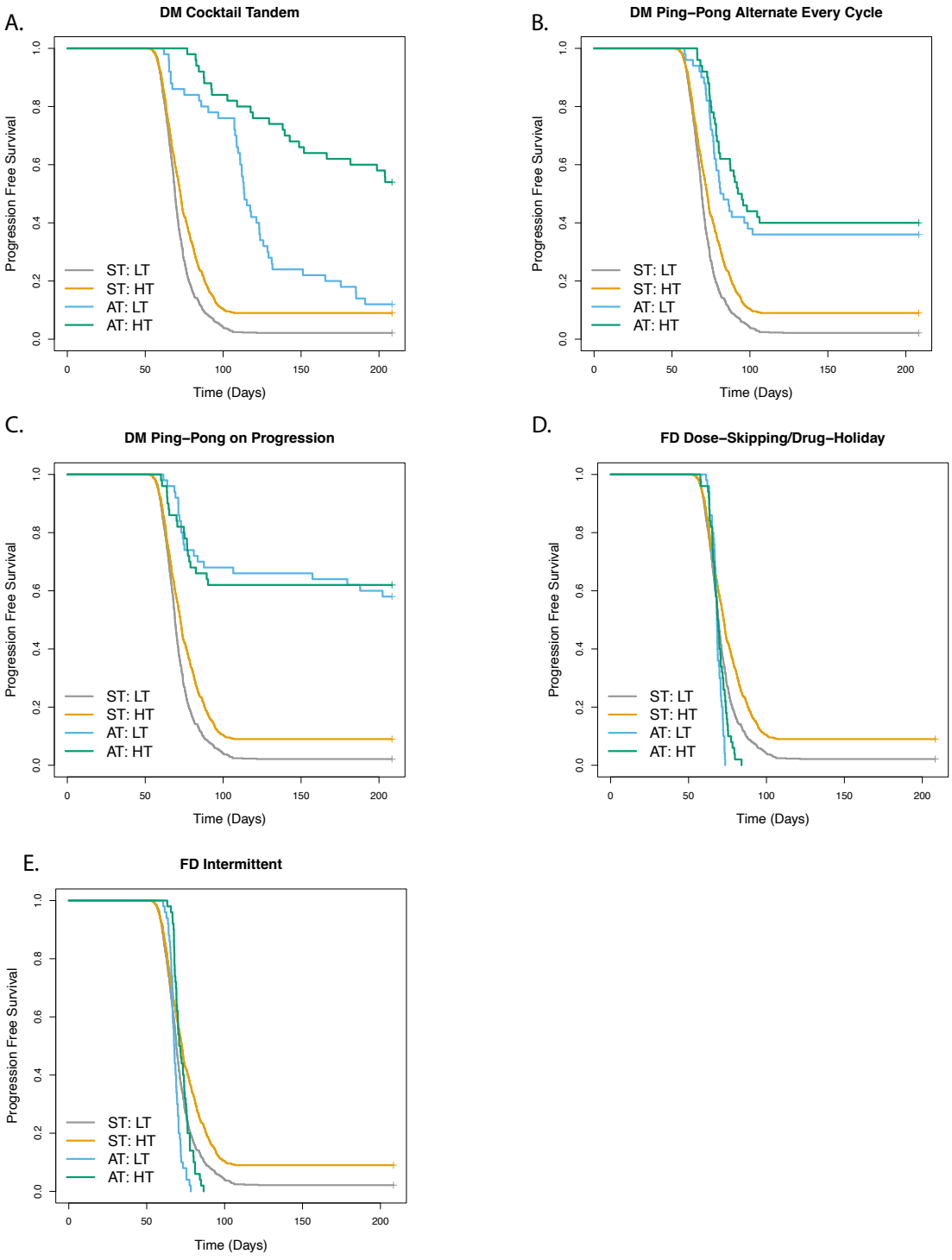

Figure 3.6: Effect of turnover on outcome of adaptive therapy with 2 drugs. The panels show the comparison of adaptive therapy (AT) versus standard treatment (ST) as turnover is varied while keeping the doubling time and net growth rate identical for (**A**) DM Cocktail Tandem, (**B**) DM Ping-Pong Alternate Every Cycle, (**C**) DM Ping-Pong on

Progression, (**D**) FD Dose-Skipping/Drug-Holiday, (**E**) FD Intermittent. For low turnover (LT) conditions, the death rate was half of the default, at 0.005/h for all cell types, while for high turnover (HT) conditions, the death rate was twice the default at 0.02/h for all cell types. Division rates were set for each cell type to keep the fitness differences (net growth rates) the same as the default conditions. The dose modulation protocols worked well regardless of the amount of turnover. High cell turnover led to statistically significantly improved TTP in ST, DM Cocktail Tandem, and though the effect size was small, in both FD protocols.

Table 3.2: Doubling time of the cell types.

Cell Types	**Doubling Time**
Doubly sensitive	13.86 h
Resistant to drug 1	23.1 h
Resistant to drug 2	23.1 h
Doubly resistant	69.3 h

Treatment with every dose modulation protocol improved time to progression, relative to ST, when cell turnover was low (DM Cocktail: HR = 0.25 [0.18–0.35, $p <$ 0.001; DM Ping-Pong Alternate: HR = 0.28 [0.19–0.40], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.14 [0.09–0.22], $p < 0.001$), and also when cell turnover was high (DM Cocktail: HR = 0.20 [0.13–0.30], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.35 [0.24–0.51], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.22 [0.14–0.34], $p <$ 0.001).

DM Cocktail Tandem protocol worked particularly well where cell turnover was high, versus when it was low (HR = 0.29 [0.17–0.49], $p < 0.001$), whereas the amount of turnover had no significant effect on the success of the other two dose modulation protocols (DM Ping-Pong Alternate: $p = 0.424$; DM Ping-Pong on Progression: $p =$ 0.834). ST also worked better when there were high levels of turnover in the tumor (HR = 0.68 [0.61–0.76], $p < 0.001$), relative to low turnover, as did the fixed dose adaptive therapy protocols (FD Intermittent: 0.39 [0.26–0.60], $p < 0.001$; FD Dose-Skipping/Drug

Holiday: HR = 0.61 [0.39–0.93], $p = 0.0234$), but again, the fixed dose adaptive therapy protocols performed worse than ST, when cell turnover was high (FD Intermittent: HR = 1.44 [1.07–1.92], $p = 0.0151$; FD Dose-Skipping/Drug Holiday: HR = 1.90 [1.42–2.55], $p < 0.001$), in addition to when cell turnover was low (FD Intermittent: HR = 1.58 [1.18–2.11], $p = 0.00213$; FD Dose-Skipping/Drug Holiday: HR = 1.60 [1.19–2.14], $p = 0.00175$).

Tumor doubling times were typically much slower than cell culture doubling times. To investigate this, we tested reducing all of the division rates and death rates by an order of magnitude. This had the effect of exposing the cells to an order of magnitude more drug before they could divide. None of the simulated tumors in any of the protocols progressed within the 200 days, used in our other experiments, but eventually they all progressed (Appendix B: **Supplementary Figure S3**). Under these much slower kinetics, most of the adaptive therapy protocols were not statistically significantly better than standard therapy, in fact, DM Cocktail was worse. Only FD Dose-Skipping/Drug Holiday performed better than standard therapy (Appendix B: **Supplementary Table S9**).

Cell Replacement Increases the TTP with Adaptive Therapy

We investigated the role of spatial structure and the ability of cells to replace their neighbors (Fig. 3.7 and Appendix B: **Supplementary Table S4**). If cells could not replace their neighbors, then they had to wait for a neighbor to die before they could reproduce in a crowded area of the tumor. Treatment worked better the more readily cells could replace their neighbors for all protocols ($p < 0.01$ and HR ≤ 0.83), with only two exceptions: for DM Ping-Pong Alternate Every Cycle, there was no significant difference between 50% and 100% replacement ($p = 0.275$), and for DM Ping-Pong on Progression,

that difference was only modestly significant, though still with a large effect size (HR = 0.40 [0.17–0.92], p= 0.031). Dose modulation AT worked better than ST ($p < 0.001$ and HR ≤ 0.32 in all cases) and there was little difference between ST and FD protocols (see Appendix B: **Supplementary Table S4** for hazard ratios, p-values, and Cox regression p-values for all comparisons). For the DM Cocktail protocol, the degree of replacement among the cells had a particularly strong effect (Fig. 3.7A), with the hazard ratio = 0.08 [0.04–0.16], $p < 0.001$ when comparing 0% vs. 50% replacement, and the hazard ratio = 0.05 [0.02–0.15], $p < 0.001$ when comparing 50% vs. 100% replacement.

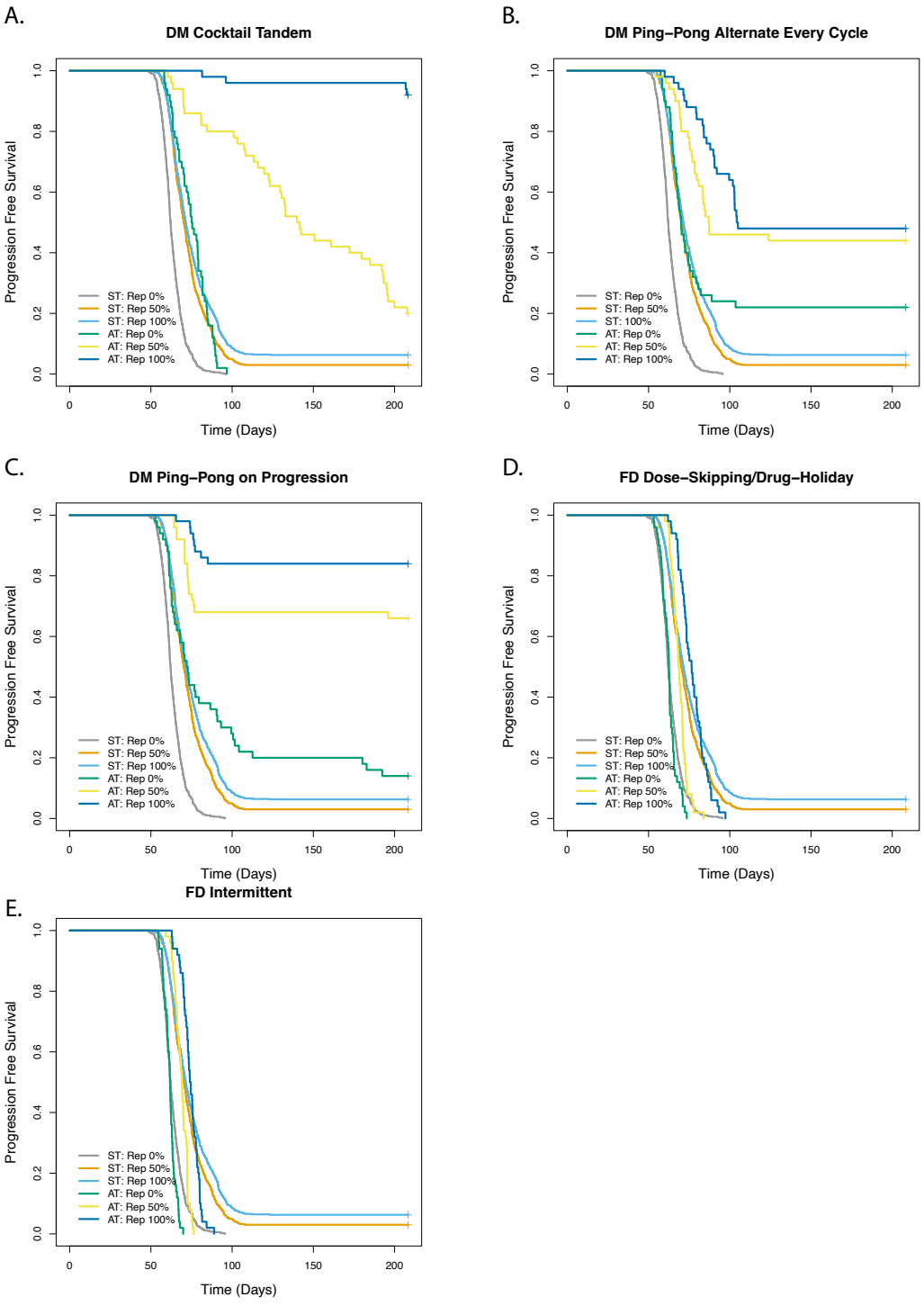

Figure 3.7: Effect of replacement on outcome of adaptive therapy with 2 drugs. The panels show the comparison of adaptive therapy (AT) versus standard treatment (ST) as the replacement parameter is varied for (**A**) DM Cocktail Tandem, (**B**) DM Ping-Pong Alternate Every Cycle, (**C**) DM Ping-Pong on Progression, (**D**) FD Dose-Skipping/Drug-

Holiday, (**E**) FD Intermittent. The replacement parameter determined the probability that a dividing cell with no empty neighbors could replace a neighbor. We tested the two extremes in which a cell can always replace a neighbor (Rep 100%), representing direct cell competition, cancer cell cannibalism, or cell death due to crowding. We represented complete contact inhibition when a cell can never replace a neighbor (Rep 0%). We also tested an intermediate value (our default) in which a dividing cell can replace its neighbor 50% of the time (Rep 50%), representing some cell death due to crowding and other forms of competition, but also some degree of contact inhibition.

Adaptive Therapy Works Better If Smaller Changes in the Tumor Burden Trigger a Change in Dose

We investigated the role of the Delta Tumor parameter that determined how much the tumor burden must change before we changed the dose in the dose modulation protocols, or skipped a dose in the FD protocols (Fig. 3.8, Appendix B: **Supplementary Figure S1, and Appendix B: Supplementary Table S5**). The dose modulation protocols were better than ST for all values of Delta Tumor (HR < 0.57 and $p < 0.001$). Treatment with DM Cocktail and Delta Tumor = 5%, achieved 100% survival and we observed no cases of progression. However, increasing the Delta Tumor value, that is, Delta Tumor = 40%, resulted in a TTP that was either significantly better than (DM Ping-Pong Alternate Every Cycle: HR = 0.67 [0.50–0.90], $p = 0.00795$), or not significantly different (DM Cocktail: $p = 0.166$; DM Ping-Pong on Progression: $p = 0.545$) from ST.

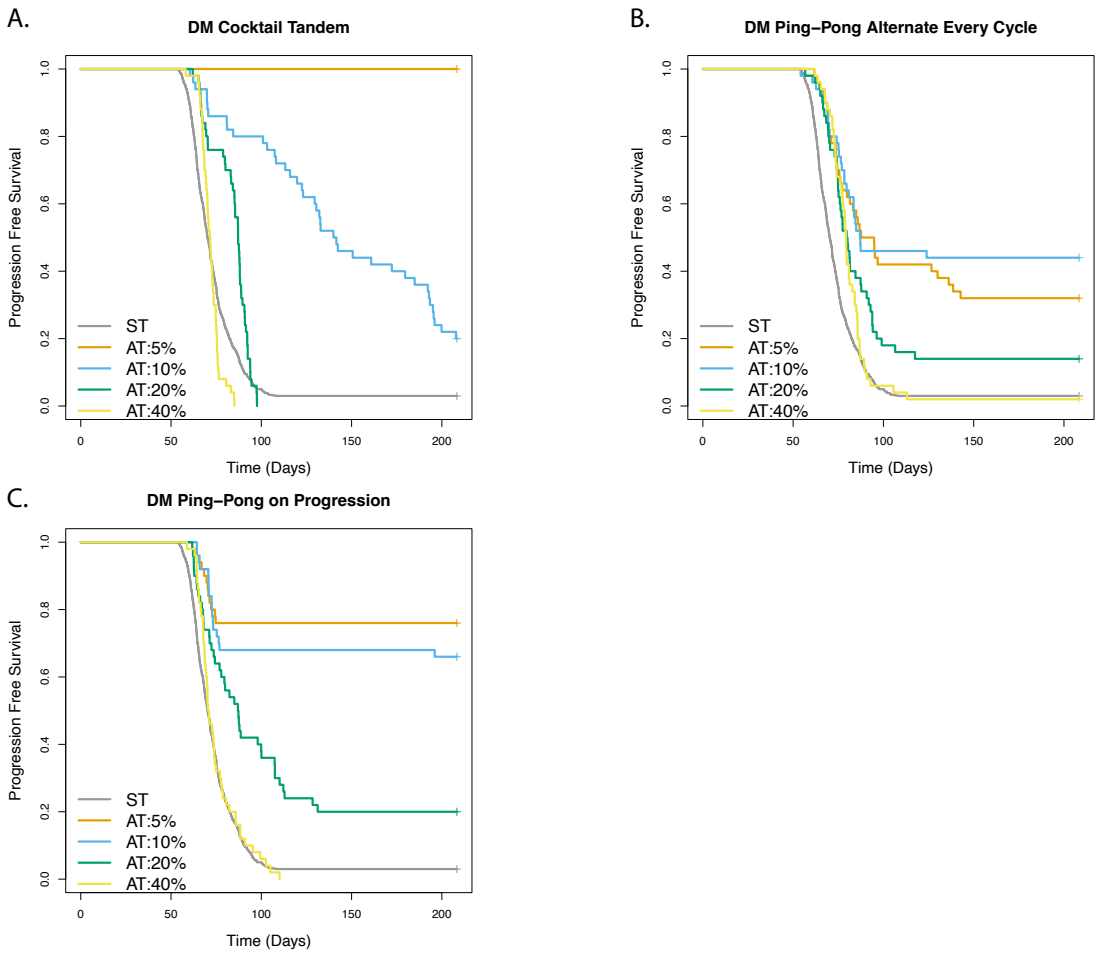

Figure 3.8: Delta Tumor is an important parameter determining outcome of dose modulation (DM) adaptive therapy. The panels show the comparison of adaptive therapy (AT) versus standard treatment (ST) as the Delta Tumor parameter is varied for (**A**) DM Cocktail Tandem, (**B**) DM Ping-Pong Alternate Every Cycle, (**C**) DM Ping-Pong on Progression. For the dose modulation (DM) protocols, Delta Tumor is the tumor measurement parameter specifying a relative value by which the tumor burden must change, compared with the last time it was measured, in order to trigger a change in drug dosage.

For the dose modulation protocols, TTP was progressively worse (HR $\geq$ 1.97 and $p \leq 0.00571$) as we increased the value of the Delta Tumor from 5 to 10 to 20%, with two exceptions where these effects were not significant: increasing Delta Tumor from 5% to 10% for DM Ping-Pong Alternate ($p = 0.397$) and DM Ping-Pong on Progression (p=

0.356). As there was no recorded case of progression for DM Cocktail with Delta Tumor = 5% we were unable to calculate a hazard ratio but the TTP was clearly better for Delta Tumor = 5% versus 10% (Fig. 3.8A, Chi Sq. $p < 0.001$).

Time to progression for treatment with the fixed dose protocols (FD Dose-Skipping/Drug Holiday and FD Intermittent) was either not significantly different or worse than treatment with ST (Appendix B: **Supplementary Figure S1A,B, Appendix B: Supplementary Table S5**). For FD Dose-Skipping/Drug Holiday, increasing Delta Tumor from 5% to 10% ($p = 0.484$), 10% to 20% ($p = 0.254$), or 20% to 40% ($p = 0.51$), did not result in any significant difference in TTP. For FD Intermittent, we stopped treatment when the tumor burden fell below the given percentage value relative to the initial tumor burden, not the last measure of tumor burden. Therefore, we investigated stopping treatment when the tumor shrank to 50% of the initial baseline for treatment initiation (the default), in addition to 80%, 90%, or 95% of the initial baseline for treatment initiation (Appendix B: **Supplementary Figure S1B**), though changing this parameter had no significant effects on TTP (50% vs. 80 [$p = 0.0767$], 80% vs. 90% [$p = 0.13$], and 90% vs. 95% [$p = 0.432$]).

For Dose Modulation Regimens, the Amount by Which the Drug Dose Is Changed (Delta Dose) Has Little Effect on the Success of Adaptive Therapy

For the dose modulation protocols, we investigated the effect of a range of Delta Dose values (25%, 50%, or 75%) which determined how much we changed the dose relative to the last application of the drug, when the tumor burden changed by Delta Tumor (Fig. 3.9 and Appendix B: **Supplementary Table S6**). In every case the TTP was improved relative to ST, that is, for Delta Dose = 25% (DM Ping-Pong Alternate: HR =

0.37 [0.26–0.53], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.13 [0.08–0.23], $p < 0.001$), Delta Dose = 50% (DM Cocktail: HR = 0.34 [0.24–0.47], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.46 [0.33–0.64], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.15 [0.09–0.25], $p < 0.001$), and Delta Dose = 75% (DM Cocktail: HR = 0.40 [0.29–0.54], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.39 [0.27–0.55], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.29 [0.20–0.44], $p < 0.001$), with the exception of DM Cocktail, where dose adjustment by Delta Dose = 25% had no significant effect relative to treatment with ST ($p = 0.355$). For both DM Ping-Pong Alternate and DM Ping-Pong on Progression, increasing the value of Delta Dose from 25 to 50% (DM Ping-Pong Alternate: $p = 0.306$; DM Ping-Pong on Progression: $p = 0.754$), or 50 to 75% (DM Ping-Pong Alternate: $p = 0.451$; DM Ping-Pong on Progression: $p = 0.0795$), did not have a significant effect on TTP. However, for DM Cocktail, changing the dose by 50% was better than changing it by either 25% or 75% (increasing Delta Tumor from 25 to 50%: HR = 0.09 [0.04–0.18], $p < 0.001$; increasing Delta Tumor from 50 to 75%: HR = 1.74 [1.13–2.67], $p = 0.0112$). These results suggest that the success of adaptive therapy is not very sensitive to change in the Delta Dose parameter, as long as it is kept above a certain threshold for DM Cocktail, where it pays to not be too conservative with this parameter.

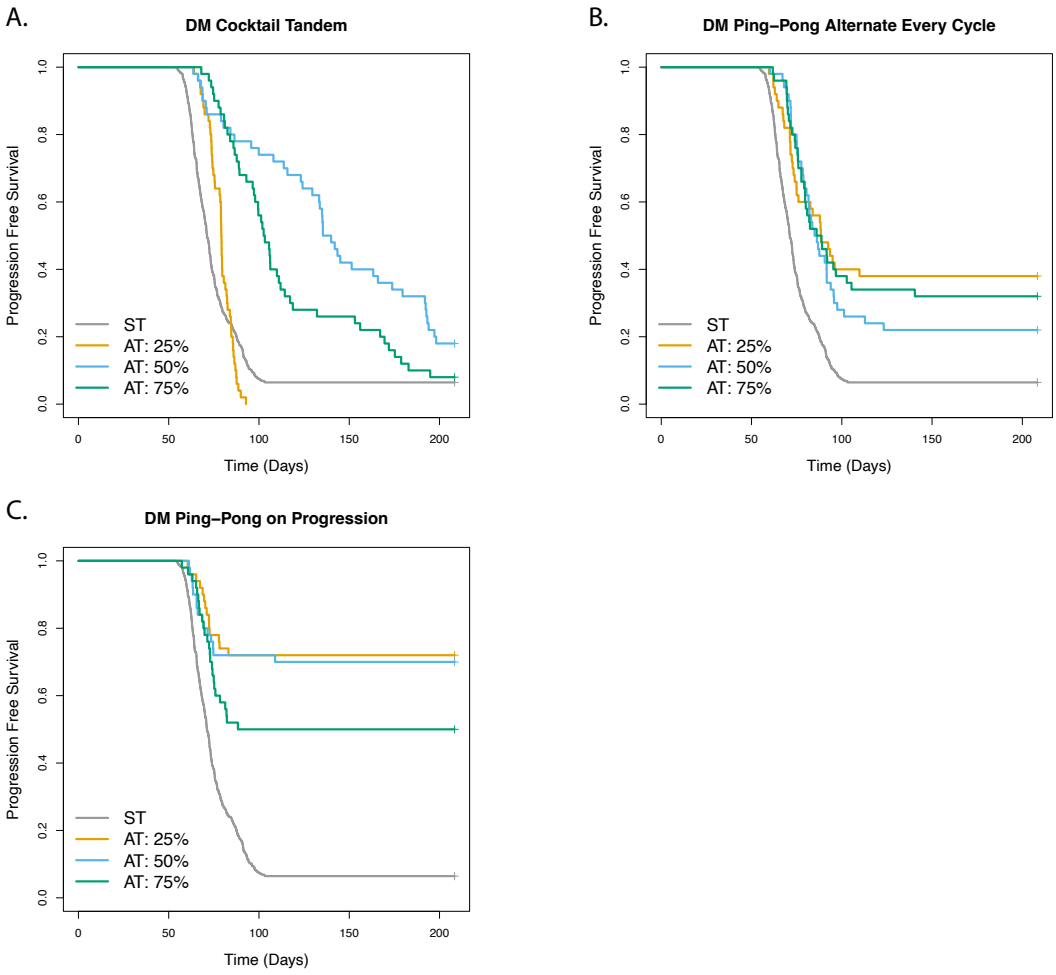

Figure 3.9: Role of the Delta Dose parameter for dose modulation (DM) adaptive therapy protocols. The panels show the comparison of adaptive therapy (AT) versus standard treatment (ST) as the Delta Dose parameter is varied for (**A**) DM Cocktail Tandem, (**B**) DM Ping-Pong Alternate Every Cycle, (**C**) DM Ping-Pong on Progression. Delta Dose is the percentage by which the drug dose is changed (increased or decreased) relative to the last time the same drug was administered. Default value is 50% for both drugs.

Dose Modulation Adaptive Therapy Works Best When Frequent Treatment Vacations Are Allowed

For the dose modulation protocols, we investigated how the stopping conditions for dosing (triggering a treatment vacation) affects TTP (Fig. 3.10 and Appendix B:

Supplementary Table S7). We note that, for the dose modulation protocols, relative to ST, it was better to stop dosing when we could still detect the tumor, e.g., at a threshold of 50% (DM Cocktail: HR = 0.25 [0.18–0.35], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.26 [0.18–0.38], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.13 [0.08–0.22], $p < 0.001$), or 80% (DM Cocktail: HR = 0.19 [0.13–0.26], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.19 [0.13–0.28], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.16 [0.11–0.25], $p < 0.001$) of the initial tumor burden. This was in contrast to waiting until the tumor had shrunk to very low or undetectable levels (as is traditional in oncology), here represented as 10% of the initial tumor burden, in which case the TTP was either worse (DM Cocktail: HR = 1.95 [1.45–2.61], $p < 0.001$; DM Ping-Pong on Progression: HR = 1.38 [1.03–1.84], $p = 0.0311$) or not significantly different than ST (DM Ping-Pong Alternate: $p = 0.277$). TTP was improved for every DM protocol when we moved from a 10% to a 50% threshold of the initial tumor burden for stopping treatment (DM Cocktail: HR = 0.05 [0.02–0.11], $p < 0.001$; DM Ping-Pong Alternate: HR = 0.25 [0.15–0.40], $p < 0.001$; DM Ping-Pong on Progression: HR = 0.13 [0.07–0.24], $p < 0.001$). However, there was no significant difference in the results comparing a 50% versus 80% threshold (DM Cocktail: $p = 0.0966$; DM Ping-Pong Alternate: $p = 0.31$; DM Ping-Pong on Progression: $p = 0.76$). Note that the dose modulation protocols did not work well when waiting for the tumor to shrink below 10% of its initial size before stopping dosing. This implies that these treatment vacations are a crucial aspect of the dose modulation protocols.

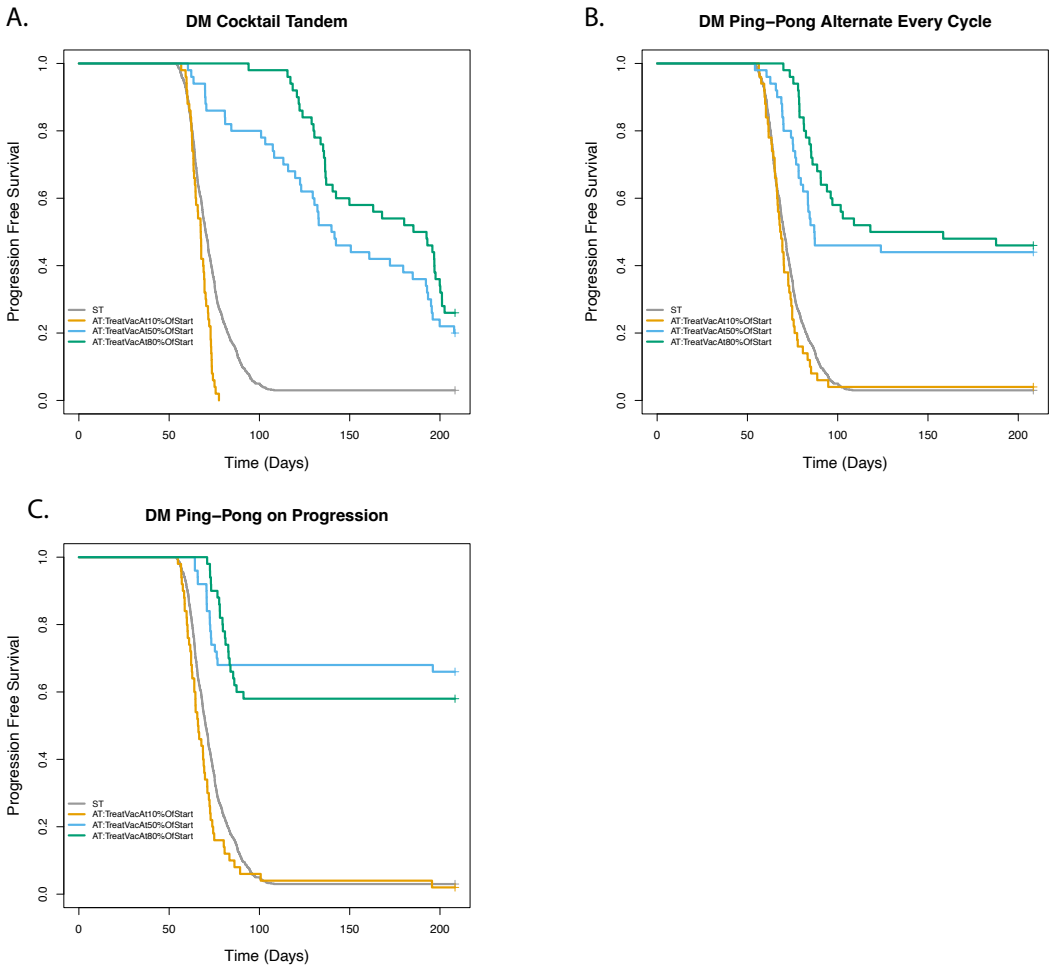

Figure 3.10: The effect of stopping treatment when the tumor burden falls below some threshold. The panels show the comparison of adaptive therapy (AT) versus standard treatment (ST) as the treatment vacation parameter is varied for (**A**) DM Cocktail Tandem, (**B**) DM Ping-Pong Alternate Every Cycle, (**C**) DM Ping-Pong on Progression. In the DM protocols, doses are adjusted as the tumor burden changes. However, a treatment vacation is triggered when the tumor burden falls below a certain threshold, resulting in no drug being administered for the treatment cycle, and treatment is resumed if the tumor regrows above that threshold. In the clinic this is often performed when the tumor is no longer detectable. Default value of the treatment vacation parameter for the DM protocols was 50% of the value at which therapy was initiated (TreatVacAt50%OfStart), that is, 25% of the carrying capacity. No statistically significant improvement in TTP was observed between 50% and 80% but 10% vs. 50% was statistically significant for all DM protocols, suggesting that we should stop dosing altogether as soon as is feasible, and not wait for the tumor to disappear.

Discussion

Design of multi-drug adaptive therapy protocols is challenging, as the number of parameters and potential protocols increases exponentially with each additional drug. However, drug combinations may afford more opportunities for better tumor control, relative to any single drug. Here, we tackled the challenge of designing multi-drug adaptive therapy protocols by investigating the simplest case of multi-drug adaptive therapy, that is, treatment with two drugs. Questions abound as to whether the treatment protocol should be sequential or concomitant (J. B. West et al. 2019a). We note that the only adaptive therapy clinical trial published to date used a fixed dose of a single drug (combined with a backbone drug held on continuously) and the published mouse experiments also used a single drug (Gatenby, Silva, Gillies, and Roy Frieden 2009; Enriquez-Navas et al. 2016). We simulated five different multi-drug adaptive therapy protocols with two drugs, and a standard-of-care standard treatment (ST) at maximum tolerated dose, under various scenarios of tumor dynamics, with the goal of finding the most promising treatment protocols.

We showed that, relative to standard of care standard treatment (ST) at maximum tolerated dose, for the default values of the parameters tested, treatment with the DM protocols increased time to progression, but the FD protocols were worse than ST (Fig. 3.4A and Appendix B: **Supplementary Table S1**). These results were consistent with preclinical adaptive therapy experiments in mice with breast cancer, where mice treated with a single drug regimen of paclitaxel, as per the dose modulation adaptive therapy (AT-1) algorithm, improved TTP in mice, while treatment as per the FD Dose-Skipping (AT-2) algorithm did not, and in fact appeared to be worse than standard therapy (STD)

for the ER+ model (Enriquez-Navas et al. 2016). In contrast, in the prostate clinical trial of adaptive therapy, the FD Intermittent protocol worked better than ST (Zhang et al. 2017). Importantly, this trial was in a relatively slow growing cancer (metastatic, castrate resistant, prostate cancer), a situation that we did not model. The dose modulation protocol, that our model suggests would have worked even better, has not been tested in any clinical trial.

Among the dose-modulation protocols, the ping-pong protocols often resulted in long term control of the tumors (Fig. 3.4A), and used only 34.0% (drug 1) and 39.8% (drug 2) for DM Ping-Pong on Progression, 32.6% (drug 1) and 66.5% (drug 2) of the amount of drug used in standard treatment, whereas the tumors eventually progressed under the Cocktail Tandem protocol, in most cases (using only 52.1% (drug 1) and 52.1% (drug 2) of the drug used in standard treatment, over the same amount of time). This was probably because the ping-pong protocols only apply one drug at a time, so there was no direct selection for doubly resistant cells. This meant that we continued to control the tumor by switching drugs. In contrast, the Cocktail Tandem protocol always applied both drugs at the same time, favoring doubly resistant cells, and eventually leading to progression.

Adaptive therapy depends on the sensitive cells being able to out-compete the resistant cells, in the absence of drug, or in the presence of low doses of drug. In our model, this fitness differential had to be more than three-fold for the DM protocols, and a five-fold difference was not sufficient to make the FD protocols work (Fig. 3.5 and Appendix B: **Supplementary Table S2**). It is possible that there is some level of fitness cost of resistance at which fixed dose protocols will work better than ST. It is

worth noting that, we and others have shown that when using models that ignore the possibility of a return to sensitivity, then FD adaptive protocols with single drugs do work better than ST (Gallaher et al. 2018; Viossat and Noble 2021; Strobl et al. 2021; Williams et al. 2018). Whether resistance generally incurs a fitness cost, and what is the magnitude of the fitness penalty incurred by the resistant cells, are open questions. In one study, MCF7 cells resistant to Doxorubicin (MCF7Dox) had a doubling time of 60 h, while sensitive MCF7 (MCF7) cells had a doubling time of 40 h (Gallaher et al. 2018). In Lotka–Volterra competition models of adaptive therapy, it is common practice to assign competition coefficients based on the assumption that resistance comes at a fitness cost (Zhang et al. 2017; J. B. West et al. 2019a). While the fitness costs we modeled were high relative to what is typically observed in organismal evolution, they were not unreasonable given observations in clonal evolution of cancer (Williams et al. 2018). Furthermore, Gatenby et al. presented calculations of the competition coefficients that best fit the data from the clinical trial (Zhang et al. 2017) and found that the cells resistant to abiraterone had a 7X lower fitness than the sensitive cells. In contrast, even in our high fitness cost case where the doubly resistant cells incurred a 5X fitness cost, the singly resistant cells only incurred a 1.7X fitness cost compared with the sensitive cells.

The amount of cell turnover had little effect on the success of adaptive therapy, except in DM Cocktail Tandem which performed better at high levels of turnover (Fig. 3.6 and Appendix B: **Supplementary Table S3**). In our previous modeling work, we underscored the importance of turnover in agent-based and mathematical models (Gallaher, Brown, and Anderson 2019; Strobl et al. 2021). Increased turnover can improve adaptive therapy if there is a fitness cost of resistance, but higher fitness costs do

not necessarily translate to an improved outcome in the absence of increased turnover (Strobl et al. 2021). However, this previous work only modeled a single drug and both the cost of resistance and cell turnover changed to fit the dynamics of the clinical data. This highlighted the possibility that these effects can occur simultaneously, and, in fact, trade off with one another. Here, we tried to disentangle the effects of fitness cost from turnover, by ensuring that the doubling time of each cell type was identical for the two scenarios (low and high turnover). In general, our model predicted that the fitness cost of resistance appeared more important than the degree of turnover (Fig. 3.5 and Fig. 3.6). We also found that the frequency of dosing relative to the cell doubling times could have dramatic effects on the success of all of the therapies (Appendix B: **Supplementary Figure S3**).

One of the most important parameters in the model was the probability that a dividing cell may replace its neighbor. In reality, it is not clear if a dividing cell can replace a neighbor. In fact, we do not know how the local density of a cancer cell affects its reproduction and survival. Stem cells are able to replace each other in a stem-cell niche (Vermeulen and Snippert 2014; Vermeulen et al. 2013), and there is evidence of cancer cell cannibalism (entosis) in which one cell kills its neighbor (Durgan and Florey 2018; Fais and Overholtzer 2018; Hamann et al. 2017). Because it is an open question regarding how cancer cells behave when there is no adjacent space available to divide, we introduced a replacement parameter in the model to represent a range of possibilities. We showed that it was possible to achieve improved TTP with dose modulation (DM) adaptive therapy protocols, relative to ST, regardless of whether a dividing cancer cell was able to replace a neighboring cell (Fig. 3.7 and Appendix B: **Supplementary Table**

S4), however dose modulation protocols worked substantially better the more that cells could replace their neighbors, which is a particular type of cell turnover.

Our results suggest that if we are to manage cancers based on their response to our therapies (i.e., Delta Tumor), we should be as sensitive as possible to changes in tumor burden, and adjust our therapy accordingly (Fig. 3.8). In practice, this may be limited by the error in measurements of tumor burden (and the frequency with which we can measure it).

How much to change the dose when the tumor grows or shrinks (Delta Dose) is a key parameter for DM protocols. We found, for a wide range of Delta Dose values (25%, 50%, or 75%) that dose modulation protocols generally resulted in an improvement in TTP relative to ST (Fig. 3.9 and Appendix B: **Supplementary Table S6**), and that there was often little difference in outcomes between the different values of Delta Dose, except in a few cases when Delta Dose was too low. These results also suggest we should not be too conservative about changing the dosages when the tumor burden changes. This is consistent with the results of a previous model using single-drug adaptive therapy, in which a dose modulation regimen with a Delta Tumor = 10% and Delta Dose = 50%, resulted in improved treatment outcome relative to continuous treatment at maximum tolerated dose (which we call standard treatment) (Gallaher et al. 2018). In that study, the investigators compared Delta Tumor = 5% and Delta Dose = 25% versus Delta Tumor = 10% and Delta Dose = 50%, and found that the latter protocol worked better (Gallaher et al. 2018). Our model suggested that Delta Tumor = 5% and Delta Dose = 50% would be even better.

Interestingly, we found that, for DM protocols to work, it was helpful for frequent treatment vacations to be incorporated in the protocols. Waiting too long to start a treatment vacation, e.g., waiting until the tumor burden fell to 10% of its initial level, resulted in a TTP that was either worse or not significantly different than ST (Fig. 3.10 and Appendix B: **Supplementary Table S7**). We found good results when we stopped dosing if the tumor only shrank to 80% of its initial level. That would not even qualify as a partial response by RECIST 1.1 criteria (Gallaher et al. 2018; Schwartz et al. 2016). This result is consistent with the current understanding that, the less drug dose we administer to a tumor, the less we select for therapeutic resistance.

Our work has some limitations. We did not explore the role of angiogenesis; three-dimensional tissue architecture composed of cancer cells, normal cells, immune cells and other stromal cells in the microenvironment of a tumor. We attempted to capture what happens in individual cross-sections of the tumor, which we assumed to be adequately perfused with capillaries, such that the drug diffusion and delivery was not limiting, and that the drug delivery was not limited by constraints imposed by tumor architecture and pressure inside the tumor. We also implicitly assumed an ability to transition freely between the four cell types, albeit driven by mutation or epigenetic modification (Brown et al. 2014; Garcia-Martinez et al. 2021), and while it does make the tumor more difficult to treat it also means that any cell that can divide can become doubly or singly resistant or sensitive with a single division. We have previously looked at the role of plasticity in adaptive therapy using a combination of in vitro/in vivo and mathematical modeling (Smalley et al. 2019), in addition to clinical data (Kim et al. 2021). In both cases, a key parameter was the switching rate between the cell types. Here,

we used an unrealistically high mutation rate to compensate for an unrealistically low cell population size. We also used a computationally efficient abstraction of cell crowding dynamics and explored a range of parameters that modeled different possibilities for those dynamics. Future efforts might consider a range of different mutation rates, extend our model to three dimensions, model cells pushing neighbors aside, include additional hallmarks of cancer, and incorporate a more realistic representation of blood vessel dynamics, in addition to nutrient and drug delivery.

Importantly, while most models predict recurrence of the tumor due to resistant cells ultimately taking over in the tumor, our results suggest it is possible to maintain indefinite control over the tumor, lending support to the idea that it is possible to convert cancer from an acute disease that inevitably leads to death to a chronic disease that can be tolerated.

Conclusions

Our results suggested that, when combining drugs in adaptive therapies, dose modulation protocols were much better than fixed dose protocols, and ping-pong protocols were probably better than applying multiple drugs at the same time. Applying one drug at a time and switching when the tumor grew (DM Ping-Pong on Progression) worked best across many parameter variations, though adjusting the dose of both drugs at the same time could also work well (DM Cocktail Tandem), especially if we had a very sensitive measure of tumor burden (Fig. 3.8A). One attractive feature of the ping-pong protocols is that only one drug is applied at a time, which may help to reduce toxicity and selection for multidrug resistance, compared with combination therapies. Furthermore, all adaptive therapy protocols reduce the amount of drug used over the same amount of time

as standard therapy. However, if adaptive therapy is successful in controlling cancer indefinitely, the total amount of drug used will eventually exceed an MTD protocol that could not control a cancer. Dose modulation protocols are particularly effective when cell competition is more intense, and when dosing of the tumor is kept to a minimum. Furthermore, in our model, adaptive therapy worked better than standard treatment only when there was a relatively large fitness cost of resistance. This suggests that developing good biomarkers for measuring cell turnover, clonal competition for space, and the fitness cost of resistance, in addition to intra-tumor heterogeneity as a proxy for the likelihood that resistant cells are already present at diagnosis, will be important for distinguishing which cancers should be treated with adaptive therapy in the future. These predictions should be tested in pre-clinical models, and if supported there, further tested in clinical trials.

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CHAPTER 4

IN SILICO INVESTIGATIONS OF ADAPTIVE THERAPY USING TWO CYTOTOXIC OR TWO CYTOSTATIC DRUGS

Abstract

While the dose modulation (DM) protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression) involves adjusting drug dosages when the tumor burden changes, the fixed-dose (FD) protocols involves administering a specific, constant dosage of the drug only when the tumor is growing (Dose-Skipping) or when the absolute tumor burden is above the baseline level until it reduces to a certain percentage of the baseline (Intermittent). Moreover, two different drugs can be administered simultaneously (cocktail), or the drugs can be switched such that only one drug is applied at a given time (ping-pong), either every cycle (ping-pong alternate every cycle) or when the tumor grows (ping-pong on progression). The dose modulation protocols work well when treated with two cytotoxic drugs, however, the ping-pong protocols (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, FD Ping-Pong Intermittent) work well when treated with two cytostatic drugs. In general, adaptive therapy, using either two cytotoxic or two cytostatic drugs works best under conditions of high competition, such as high fitness cost, high replacement rates, and high turnover, although treatment using two cytostatic drugs works best under low turnover in many cases. Adaptive therapy works best when drug dosages are changed as soon as a change in tumor burden is detected, and it is best to pause treatment sooner rather than later when the tumor is shrinking. Adaptive therapy

works best when an intermediate level of drug dosage is used, and both treatment with too little or too much drug leads to poor survival outcome.

Introduction

In chapter 3, I have investigated multi-drug adaptive therapy protocols for treatment using two drugs. Because the drugs investigated worked by killing the cells, the anti-cancer drugs would be expected to have a cytotoxic mechanism of action, killing cells directly. Cytostatic drugs, on the other hand, work by inhibiting cellular division. An interesting question that arises is, would the survival outcome be any different for adaptive therapy using two cytostatic drugs? In this chapter, I have undertaken to answer this question by investigating seven different adaptive therapy protocols and standard treatment at maximum tolerated dose, for treatment using either two cytotoxic drugs, or two cytostatic drugs, under a wide variety of different scenarios of cell kinetics and treatment settings.

Materials and Methods

I have used the same extensions to the original model (Thomas et al. 2022) that are described in Chapter 2, including: the definition of progression (see *Observation*), the implementation of cytostatic drugs (see *Cell Death* and *Cell Division*). However, here we have 4 cell types, as in Chapter 3, with doubly sensitive cells, cells resistant to one drug but not the other, and doubly resistant cells. The changes to the original model (Thomas et al. 2022) are as follows:

In section 2.4.11 (Observation), the change that have been made to the definition of progression, as described in Chapter 2 (see *observation*), also applies here.

In section 2.7.1 (Cell Death), for treatment with two cytotoxic drugs, nothing has changed. For treatment using two cytostatic drugs, the equation for probability of cell death is as follows: Probability of cell death for a particular cell type per hour=background death probability of that particular cell type per hour.

In section 2.7.2 (Cell Division), for treatment with two cytotoxic drugs, nothing has changed. For treatment with two cytostatic drugs, probability of cell division per hour=background division probability per hour-S1*[Drug1]*$\mathbf{\Psi}$1- S2*[Drug2]*$\mathbf{\Psi}$2, where S1 and S2 are the binary indicator variables for the cell's sensitivity to drugs 1 and 2, respectively, such that a value of 1 indicates sensitivity, while a value of 0 indicates resistance to the particular drug. [Drug1] and [Drug 2] being the concentrations of those drugs (non-negative real values), and $\mathbf{\Psi}$1 and $\mathbf{\Psi}$2 being the drug potency of those drugs (non-negative real values), quantified as the probability of inhibition in cell division per unit drug concentration per hour.

In section 2.7.6 (Drug Protocols), I investigated two additional treatment protocols—FD Ping-Pong Dose-Skipping and FD Ping-Pong Intermittent—as well as changed the nomenclature as follows:

FD Cocktail Dose-Skipping: It is identical to FD Dose-Skipping/Drug Holiday, which administers both the drugs, Drug1 and 2, in a cocktail formulation.

FD Ping-Pong Dose-Skipping: It is similar to FD Cocktail Dose-Skipping, other than Drug 1 and Drug 2 are being switched every treatment cycle, with the response of a particular drug being evaluated based on the how the tumor responded to that same drug last time it was administered.

FD Cocktail Intermittent: It is identical to FD Intermittent, except that Drugs 1 and 2 are administered as a cocktail formulation at 100% of the MTD (previously I used 75% of the MTD).

FD Ping-Pong Intermittent: It is similar to FD Cocktail Intermittent, other than the drugs are switched every time the tumor climbs back to 100% or more of the baseline tumor burden at which treatment was initiated.

Results

Cytotoxic and Cytostatic Therapies

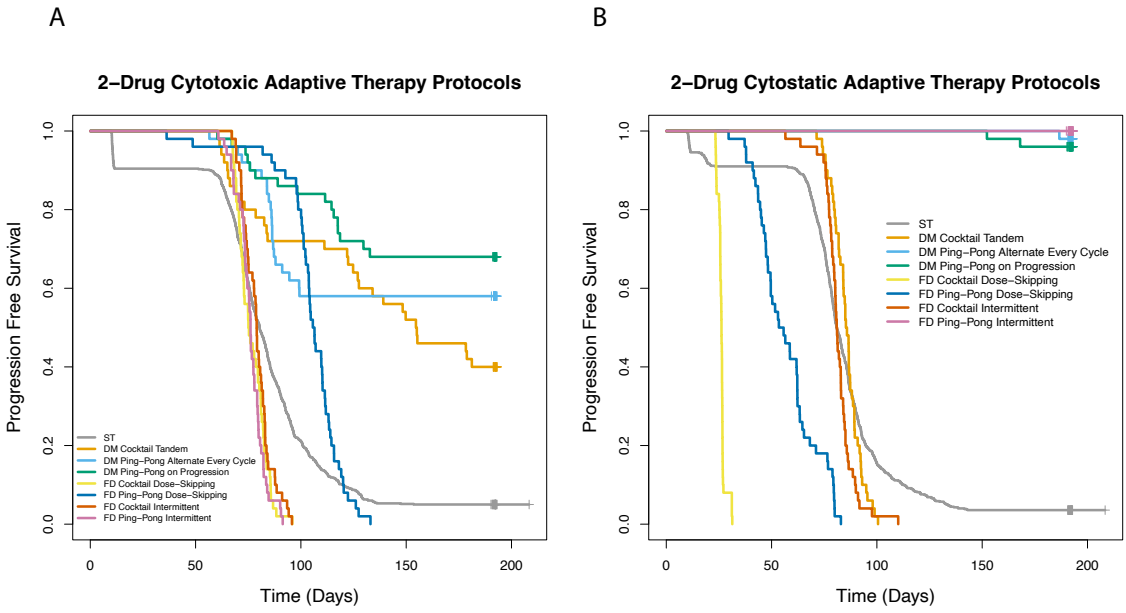

Figure 4.1: Adaptive therapy using two cytotoxic or two cytostatic drugs. Single-drug adaptive therapy protocols comparing standard treatment (ST) versus three different adaptive therapy protocols, dose modulation, dose-skipping, and intermittent using a single cytotoxic drug (Fig. 4.1A), or a single cytostatic drug (Fig. 4.1B).

For treatment using 2 cytotoxic drugs, all the dose modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on

Progression), as well as FD Ping-Pong Dose-Skipping with a relatively low effect size as measured by the hazard ratio work well (Fig. 4.1A, Table 4.1). For treatment using 2 cytostatic drugs, all the ping-pong protocols work well (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, FD Ping-Pong Intermittent) except the dose-skipping (Fig. 4.1B, Table 4.1).

Table 4.1: Adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
Default	Standard Treatment	0.27	0.19-0.39	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
Default	Standard Treatment	0.21	0.13-0.32	<0.001
DM Ping-Pong on Progression (cytotoxic)				
Default	Standard Treatment	0.13	0.08-0.21	<0.001
FD Cocktail Dose-Skipping (cytotoxic)				
Default	Standard Treatment	1.8	1.4-2.5	<0.001
FD Ping-Pong Dose-Skipping (cytotoxic)				
Default	Standard Treatment	0.56	0.42-0.74	<0.001
FD Cocktail Intermittent (cytotoxic)				
Default	Standard Treatment	1.6	1.2-2.1	<0.01

FD Ping-Pong Intermittent (cytotoxic)				
Default	Standard Treatment	1.9	1.4-2.5	<0.001
DM Cocktail Tandem (cytostatic)				
Default	Standard Treatment			Not Significant
DM Ping-Pong Alternated Every Cycle (cytostatic)				
Default	Standard Treatment	0.006	0.001-0.043	<0.001
DM Ping-Pong on Progression (cytostatic)				
Default	Standard Treatment	0.01	0.003-0.048	<0.001
FD Cocktail Dose-Skipping (cytostatic)				
Default	Standard Treatment	14.7	10.0-21.6	<0.001
FD Ping-Pong Dose-Skipping (cytostatic)				
Default	Standard Treatment	5.3	3.9-7.2	<0.001
FD Cocktail Intermittent (cytostatic)				
Default	Standard Treatment	1.4	1.0-1.8	<0.05
FD Ping-Pong Intermittent (cytostatic)				
Default	Standard Treatment	~0		

The Effect of Fitness Costs of Resistance

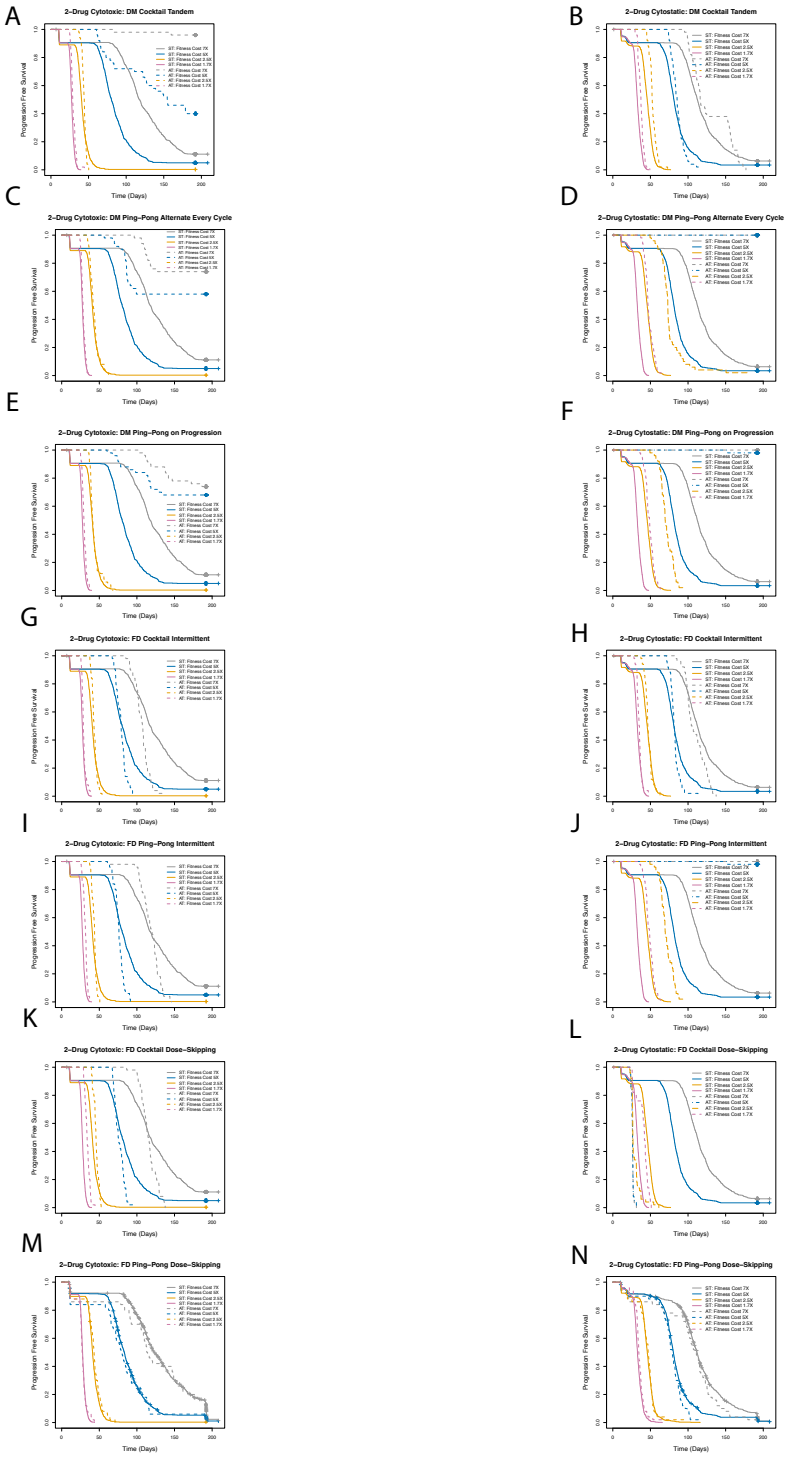

Figure 4.2. Effect of fitness cost parameter on the outcome of adaptive therapy using two cytotoxic or two cytostatic drugs. Survival outcome for treatment as per the dose modulation protocol (Fig. 4.2A, Fig. 4.2B), dose-skipping (Fig. 4.2C, Fig. 4.2D), or intermittent (Fig. 4.2E, Fig. 4.2F) under fitness cost of 1.7-fold, 2.5-fold, 5-fold, or 7-fold

relative to standard treatment for treatment using either a single cytotoxic (Fig. 4.2A, 4.2C, 4.2E), or a single cytostatic drug (Fig. 4.2B, 4.2D, 4.2F).

For treatment using two cytotoxic drugs, all the dose-modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression) work well under conditions of 5-fold or 7-fold fitness cost, and some work well even under conditions of 1.7-fold and 2.5-fold fitness cost, improving survival outcome relative to standard treatment (Fig. 4.2, Table 4.2); FD Ping-Pong works well under all fitness cost values (1.7-fold, 2.5-fold, 5-fold, 7-fold) tested (Table 4.2). For treatment using two cytostatic drugs, the ping-pong protocols (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, FD Ping-Pong Intermittent) work well under all values of fitness cost (Table 4.2); two exceptions can be noted to this general trend: FD Dose-Skipping under 5-fold or 7-fold fitness cost. In general, for treatment with either two cytotoxic or two cytostatic drugs, for the protocols that work, higher fitness cost values lead to improved survival outcome relative to low fitness cost as reflected in relatively low hazard ratios under conditions of higher fitness cost (Table 4.2).

Table 4.2: Effect of fitness cost parameter on the outcome of adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.62	0.46-0.82	<0.01
2.5-fold fitness cost	Standard Treatment			Not Significant

5-fold fitness cost	Standard Treatment	0.27	0.19-0.39	<0.001
7-fold fitness cost	Standard Treatment	0.02	0.005-0.075	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
1.7-fold fitness cost	Standard Treatment			Not Significant
2.5-fold fitness cost	Standard Treatment			Not Significant
5-fold fitness cost	Standard Treatment	0.21	0.13-0.32	<0.001
7-fold fitness cost	Standard Treatment	0.15	0.09-0.26	<0.001
DM Ping-Pong on Progression (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.57	0.43-0.76	<0.001
2.5-fold fitness cost	Standard Treatment			Not Significant
5-fold fitness cost	Standard Treatment	0.13	0.08-0.21	<0.001
7-fold fitness cost	Standard Treatment	0.14	0.08-0.24	<0.001
FD Cocktail Dose-Skipping (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.23	0.17-0.32	<0.001
2.5-fold fitness cost	Standard Treatment	0.67	0.50-0.90	<0.01
5-fold fitness cost	Standard Treatment	1.8	1.4-2.5	<0.001
7-fold fitness cost	Standard Treatment	1.6	1.2-2.2	<0.01

FD Ping-Pong Dose-Skipping (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.18	0.13-0.25	<0.001
2.5-fold fitness cost	Standard Treatment	0.45	0.34-0.60	<0.001
5-fold fitness cost	Standard Treatment	0.56	0.42-0.74	<0.001
7-fold fitness cost	Standard Treatment	0.37	0.26-0.54	<0.001
FD Cocktail Intermittent (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.65	0.48-0.87	<0.01
2.5-fold fitness cost	Standard Treatment			Not Significant
5-fold fitness cost	Standard Treatment	1.6	1.2-2.1	<0.01
7-fold fitness cost	Standard Treatment	2.3	1.7-3.1	<0.001
FD Ping-Pong Intermittent (cytotoxic)				
1.7-fold fitness cost	Standard Treatment	0.43	0.32-0.57	<0.001
2.5-fold fitness cost	Standard Treatment			Not Significant
5-fold fitness cost	Standard Treatment	1.9	1.4-2.5	<0.001
7-fold fitness cost	Standard Treatment	1.5	1.1-2.0	<0.05
DM Cocktail Tandem (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.50	0.37-0.66	<0.001

2.5-fold fitness cost	Standard Treatment	0.47	0.35-0.63	<0.001
5-fold fitness cost	Standard Treatment			Not Significant
7-fold fitness cost	Standard Treatment			Not Significant
DM Ping-Pong Alternated Every Cycle (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.07	0.05-0.11	<0.001
2.5-fold fitness cost	Standard Treatment	0.08	0.06-0.12	<0.001
5-fold fitness cost	Standard Treatment	~0		
7-fold fitness cost	Standard Treatment	~0		
DM Ping-Pong on Progression (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.06	0.04-0.10	<0.001
2.5-fold fitness cost	Standard Treatment	0.10	0.07-0.14	<0.001
5-fold fitness cost	Standard Treatment	0.006	0.001-0.042	<0.001
7-fold fitness cost	Standard Treatment	~0		
FD Cocktail Dose-Skipping (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.22	0.16-0.31	<0.001
2.5-fold fitness cost	Standard Treatment	4.0	2.9-5.4	<0.001
5-fold fitness cost	Standard Treatment	13.8	9.4-20.2	<0.001

7-fold fitness cost	Standard Treatment	13.8	9.4-20.2	<0.001
FD Ping-Pong Dose-Skipping (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.13	0.08-0.18	<0.001
2.5-fold fitness cost	Standard Treatment	0.35	0.25-0.48	<0.001
5-fold fitness cost	Standard Treatment	6.9	5.0-9.5	<0.001
7-fold fitness cost	Standard Treatment	13.6	9.3-19.8	<0.001
FD Cocktail Intermittent (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.72	0.54-0.96	<0.05
2.5-fold fitness cost	Standard Treatment			Not Significant
5-fold fitness cost	Standard Treatment			Not Significant
7-fold fitness cost	Standard Treatment	1.6	1.2-2.1	<0.01
FD Ping-Pong Intermittent (cytostatic)				
1.7-fold fitness cost	Standard Treatment	0.07	0.05-0.11	<0.001
2.5-fold fitness cost	Standard Treatment	0.10	0.07-0.15	<0.001
5-fold fitness cost	Standard Treatment	0.006	0.001-0.042	<0.001
7-fold fitness cost	Standard Treatment	~0		

Cell Competition

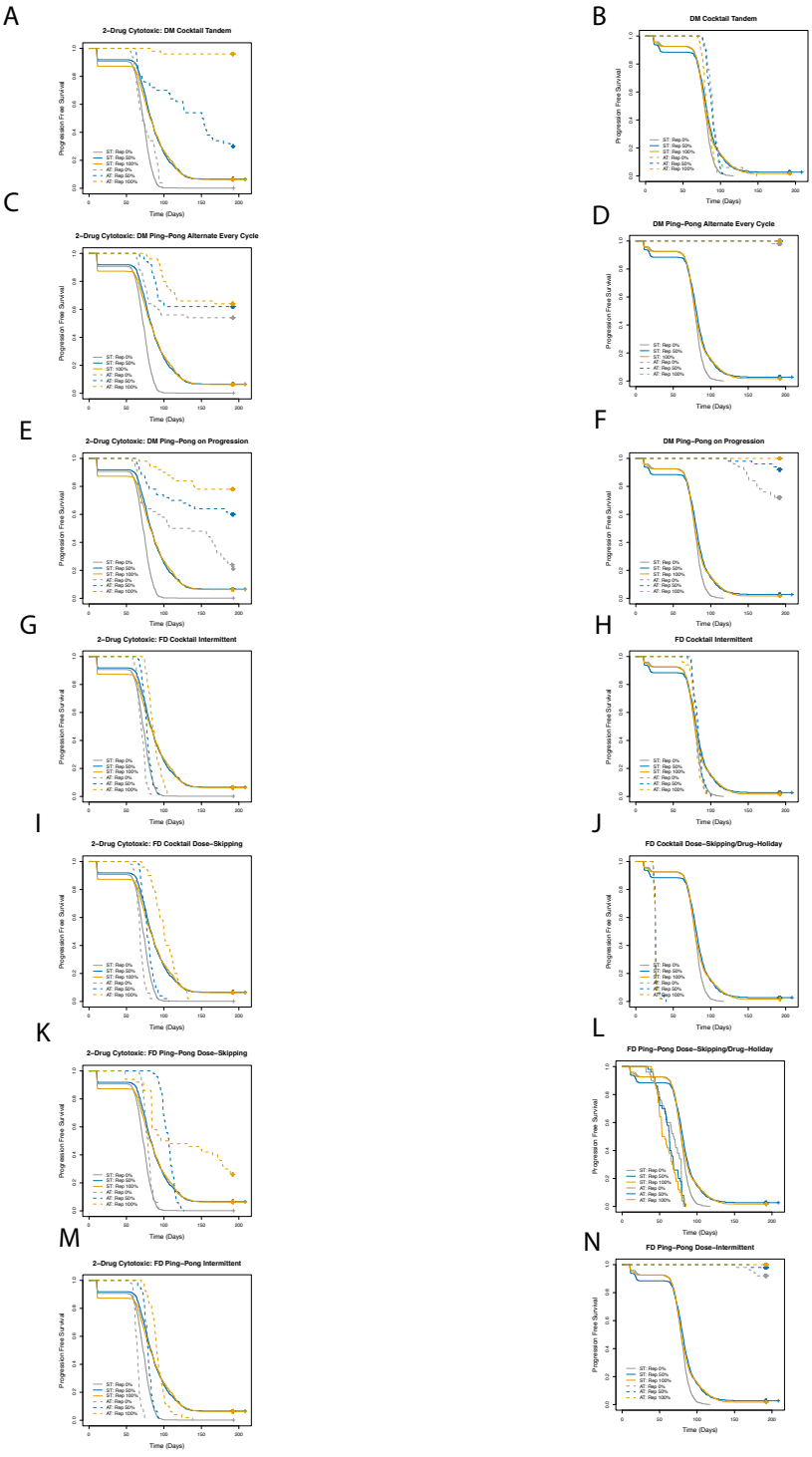

Figure 4.3. Effect of replacement parameter on outcome of adaptive therapy using two cytotoxic or two cytostatic drugs. Treatment as per the dose modulation protocol (Fig.

4.3A, 4.3B), dose-skipping protocol (Fig. 4.3C, Fig. 4.4D), or intermittent (Fig. 4.3E,Fig. 4.3F), relative to standard treatment under conditions of 0%, 50%, or 100% replacement using either a single cytotoxic (Fig. 4.3A,4.3C, 4.3E), or a single cytostatic drug (Fig. 4.3B, Fig. 4.3D, Fig. 4.3F).

For treatment using two cytotoxic drugs, all the dose-modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on progression) as well as FD Ping-Pong Dose-Skipping work well under all conditions of replacement rate tested, that is, 0%, 50%, or 100% replacement. For treatment with two cytostatic drugs, the ping-pong protocols (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, FD Ping-Pong Intermittent) work well under all conditions of replacement tested here, with the exception of FD Ping-Pong Intermittent at 0% replacement where the effect was not significant. In general, when a protocol works, for either treatment using two cytotoxic or two cytostatic drugs, 100% replacement works best, followed by 50% replacement, and 0% replacement works worst, as reflected in the relatively low hazard ratios for higher replacement rates (Fig. 4.3, Table 4.3). Some protocols work so well (such as some of the ping-protocols using two cytostatic drugs) that the percent of replacement doesn't matter as it was able to control the tumor under all conditions (Fig. 4.3D,4.3F).

Table 4.3: Effect of replacement parameter on outcome of adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
0% replacement	Standard Treatment	0.64	0.48-0.86	<0.01

50% replacement	Standard Treatment	0.37	0.26-0.52	<0.001
100% replacement	Standard Treatment	0.01	0.004-0.060	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
0% replacement	Standard Treatment	0.13	0.08-0.20	<0.001
50% replacement	Standard Treatment	0.19	0.12-0.30	<0.001
100% replacement	Standard Treatment	0.16	0.10-0.25	<0.001
DM Ping-Pong on Progression (cytotoxic)				
0% replacement	Standard Treatment	0.16	0.11-0.24	<0.001
50% replacement	Standard Treatment	0.19	0.12-0.30	<0.001
100% replacement	Standard Treatment	0.09	0.05-0.16	<0.001
FD Cocktail Dose-Skipping (cytotoxic)				
0% replacement	Standard Treatment	1.9	1.4-2.5	<0.001
50% replacement	Standard Treatment	1.8	1.3-2.4	<0.001
100% replacement	Standard Treatment	0.72	0.54-0.97	<0.05
FD Ping-Pong Dose-Skipping (cytotoxic)				
0% replacement	Standard Treatment	0.70	0.52-0.93	<0.05
50% replacement	Standard Treatment	0.64	0.48-0.85	<0.01

100% replacement	Standard Treatment	0.44	0.31-0.61	<0.001
FD Cocktail Intermittent (cytotoxic)				
0% replacement	Standard Treatment	1.7	1.2-2.2	<0.001
50% replacement	Standard Treatment	2.0	1.5-2.7	<0.001
100% replacement	Standard Treatment			Not Significant
FD Ping-Pong Intermittent (cytotoxic)				
0% replacement	Standard Treatment	3.3	2.4-4.4	<0.001
50% replacement	Standard Treatment	1.8	1.4-2.4	<0.001
100% replacement	Standard Treatment			Not Significant
DM Cocktail Tandem (cytostatic)				
0% replacement	Standard Treatment	0.41	0.30-0.55	<0.001
50% replacement	Standard Treatment			Not Significant
100% replacement	Standard Treatment			Not Significant
DM Ping-Pong Alternated Every Cycle (cytostatic)				
0% replacement	Standard Treatment	~0		
50% replacement	Standard Treatment	~0		
100% replacement	Standard Treatment	~0		

DM Ping-Pong on Progression (cytostatic)				
0% replacement	Standard Treatment	~0		
50% replacement	Standard Treatment	0.02	0.008-0.058	<0.001
100% replacement	Standard Treatment	~0		
FD Cocktail Dose-Skipping (cytostatic)				
0% replacement	Standard Treatment	19.1	12.7-28.7	<0.001
50% replacement	Standard Treatment	10.5	7.3-15.1	<0.001
100% replacement	Standard Treatment	19.1	12.7-28.6	<0.001
FD Ping-Pong Dose-Skipping (cytostatic)				
0% replacement	Standard Treatment	2.8	2.1-3.8	<0.001
50% replacement	Standard Treatment	3.9	2.9-5.3	<0.001
100% replacement	Standard Treatment	4.6	3.4-6.2	<0.001
FD Cocktail Intermittent (cytostatic)				
0% replacement	Standard Treatment			Not Significant
50% replacement	Standard Treatment			Not Significant
100% replacement	Standard Treatment			Not Significant
FD Ping-Pong Intermittent (cytostatic)				
0% replacement	Standard Treatment			Not Significant

50% replacement	Standard Treatment	0.01	0.001-0.039	<0.001
100% replacement	Standard Treatment	~0		

Cell Turnover

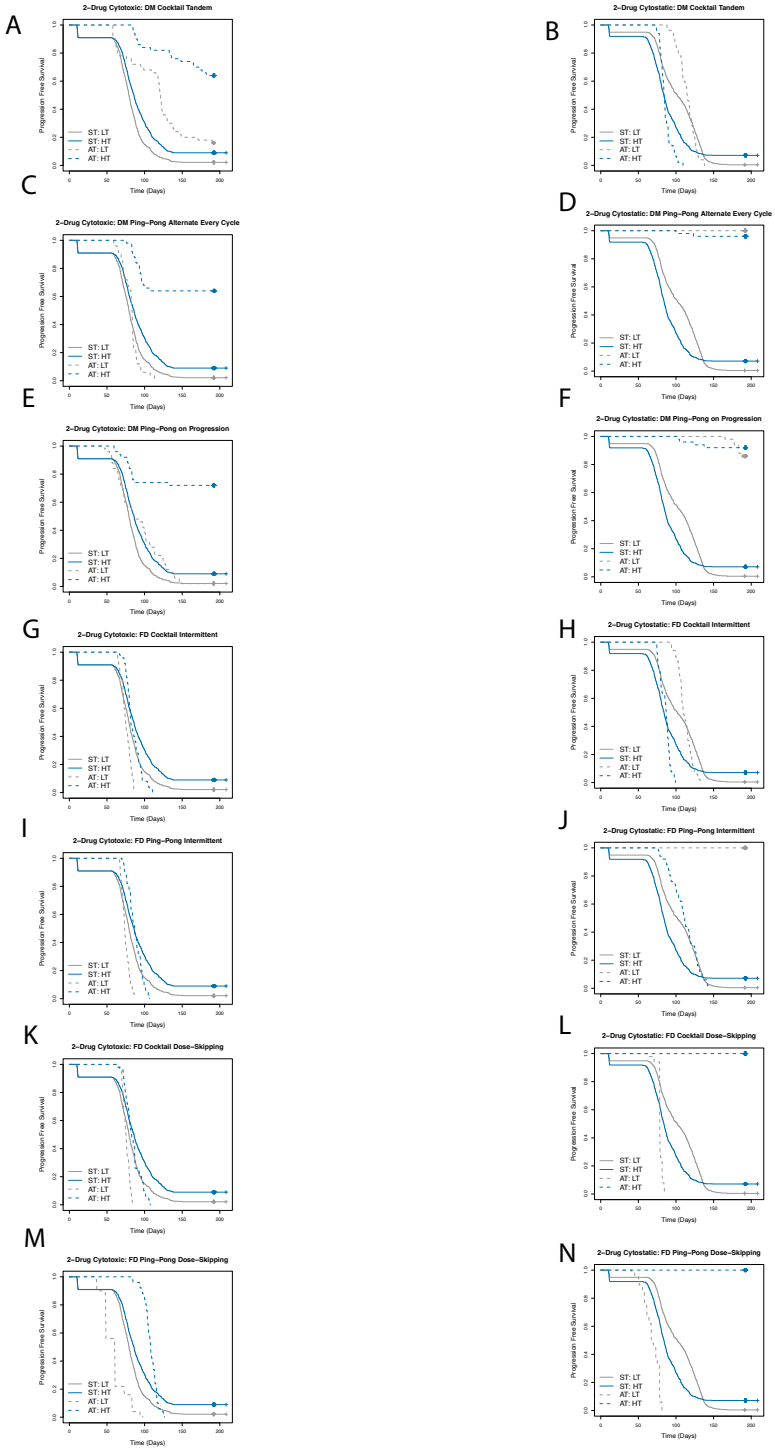

Figure 4.4. Effect of turnover on outcome of adaptive therapy using two cytotoxic or two cytostatic drugs. Survival outcome for treatment as per the dose modulation protocol (Fig. 4.4A, Fig. 4.4B), dose-skipping protocol (Fig. 4.4C, Fig. 4.4D), or intermittent (Fig. 4.4E, Fig. 4.4F) using a single cytotoxic (Fig. 4.4A, 4.4C, 4.4E) or a single cytostatic

drug (Fig. 4.4B, 4.4D, 4.4F) under conditions of low turnover (LT) or high turnover (HT), relative to standard treatment under those conditions.

For treatment using two cytotoxic drugs, the dose modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression), as well as FD Ping-Pong Dose-Skipping works well under conditions of high turnover, some also working under low turnover conditions. In contrast, for treatment using two cytostatic drugs, the ping-pong protocols (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, FD Ping-Pong Intermittent) works better under low turnover conditions; however, as an exception, FD Ping-Pong Dose-Skipping works best under high turnover conditions (Fig. 4.4L, 4.4N, Table 4.4). In general, cytostatic drugs tend to work better when turnover is low, relative to when turnover is high, including the standard treatment at maximum tolerated dose, as indicated by the low hazard ratios under low turnover conditions (Table 4.4). For cytotoxic drugs, adaptive therapy works better in tumors with high turnover (Fig. 4.4).

Table 4.4: Effect of turnover on outcome of adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
Low Turnover	Standard Treatment	0.34	0.25-0.47	<0.001
High Turnover	Standard Treatment	0.17	0.10-0.27	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				

Low Turnover	Standard Treatment			Not Significant
High Turnover	Standard Treatment	0.20	0.12-0.31	<0.001
DM Ping-Pong on Progression (cytotoxic)				
Low Turnover	Standard Treatment	0.67	0.50-0.89	<0.01
High Turnover	Standard Treatment	0.15	0.09-0.26	<0.001
FD Cocktail Dose-Skipping (cytotoxic)				
Low Turnover	Standard Treatment	1.9	1.4-2.6	<0.001
High Turnover	Standard Treatment	1.5	1.1-2.0	<0.01
FD Ping-Pong Dose-Skipping (cytotoxic)				
Low Turnover	Standard Treatment	3.4	2.6-4.6	<0.001
High Turnover	Standard Treatment	0.68	0.51-0.90	<0.01
FD Cocktail Intermittent (cytotoxic)				
Low Turnover	Standard Treatment	1.8	1.4-2.5	<0.001
High Turnover	Standard Treatment	1.5	1.1-2.0	<0.01
FD Ping-Pong Intermittent (cytotoxic)				
Low Turnover	Standard Treatment	2.0	1.5-2.7	<0.001
High Turnover	Standard Treatment	1.3	1.0-1.8	<0.05
DM Cocktail Tandem (cytostatic)				

Low Turnover	Standard Treatment			Not Significant
High Turnover	Standard Treatment			Not Significant
DM Ping-Pong Alternated Every Cycle (cytostatic)				
Low Turnover	Standard Treatment	~0		
High Turnover	Standard Treatment	0.02	0.004-0.062	<0.001
DM Ping-Pong on Progression (cytostatic)				
Low Turnover	Standard Treatment	0.02	0.01-0.05	<0.001
High Turnover	Standard Treatment	0.03	0.01-0.08	<0.001
FD Cocktail Dose-Skipping (cytostatic)				
Low Turnover	Standard Treatment	4.3	3.1-5.9	<0.001
High Turnover	Standard Treatment	~0		
FD Ping-Pong Dose-Skipping (cytostatic)				
Low Turnover	Standard Treatment	8.1	5.9-11.2	<0.001
High Turnover	Standard Treatment	~0		
FD Cocktail Intermittent (cytostatic)				
Low Turnover	Standard Treatment			Not Significant
High Turnover	Standard Treatment			Not Significant
FD Ping-Pong Intermittent (cytostatic)				

Low Turnover	Standard Treatment	~0		
High Turnover	Standard Treatment	0.57	0.43-0.76	<0.001

When to Adjust the Dose of the Drug

For treatment using two cytotoxic drugs, all the dose-modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression) works well when Delta Tumor=5%, 10%, or 20% (Fig. 4.5, Table 4.5). For treatment using two cytostatic drugs, the ping-pong protocols (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression) work well under Delta Tumor=5%, 10%, 20%, or 40% (Fig. 4.5, Table 4.5). In general, for both cytotoxic and cytostatic drugs changing the dose when the smallest change in tumor burden is detected works best (e.g., Delta Dose=5%) (Fig. 4.5, Table 4.5). In fact, with a delta dose of 5%, for the first time we observe that DM Cocktail Tandem with cytostatic drugs works well (Fig. 4.5B, Table 4.5).

Table 4.5: Effect of the delta tumor parameter on determining the outcome of adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
Delta Tumor=5%	Standard Treatment	~0		

Delta Tumor=10%	Standard Treatment	0.28	0.20-0.40	<0.001
Delta Tumor=20%	Standard Treatment			Not Significant
Delta Tumor=40%	Standard Treatment	3.4	2.6-4.6	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
Delta Tumor=5%	Standard Treatment	0.18	0.11-0.28	<0.001
Delta Tumor=10%	Standard Treatment	0.21	0.13-0.32	<0.001
Delta Tumor=20%	Standard Treatment	0.37	0.26-0.53	<0.001
Delta Tumor=40%	Standard Treatment			Not Significant
DM Ping-Pong on Progression (cytotoxic)				
Delta Tumor=5%	Standard Treatment	0.29	0.21-0.41	<0.001
Delta Tumor=10%	Standard Treatment	0.13	0.08-0.22	<0.001
Delta Tumor=20%	Standard Treatment	0.27	0.19-0.40	<0.001
Delta Tumor=40%	Standard Treatment	1.4	1.0-1.8	<0.05
FD Cocktail Dose-Skipping (cytotoxic)				
Delta Tumor=5%	Standard Treatment	1.6	1.2-2.1	<0.01
Delta Tumor=10%	Standard Treatment	1.9	1.4-2.5	<0.001
Delta Tumor=20%	Standard Treatment	1.7	1.3-2.3	<0.001

Delta Tumor=40%	Standard Treatment	2.5	1.9-3.3	<0.001
FD Ping-Pong Dose-Skipping (cytotoxic)				
Delta Tumor=5%	Standard Treatment	0.55	0.42-0.73	<0.001
Delta Tumor=10%	Standard Treatment	0.58	0.44-0.77	<0.001
Delta Tumor=20%	Standard Treatment	0.63	0.48-0.84	<0.01
Delta Tumor=40%	Standard Treatment	0.70	0.52-0.95	<0.05
DM Cocktail Tandem (cytostatic)				
Delta Tumor=5%	Standard Treatment	0.22	0.16-0.31	<0.001
Delta Tumor=10%	Standard Treatment			Not Significant
Delta Tumor=20%	Standard Treatment			Not Significant
Delta Tumor=40%	Standard Treatment			Not Significant
DM Ping-Pong Alternated Every Cycle (cytostatic)				
Delta Tumor=5%	Standard Treatment	0.02	0.01-0.05	<0.001
Delta Tumor=10%	Standard Treatment	0.01	0.001-0.039	<0.001
Delta Tumor=20%	Standard Treatment	~0		
Delta Tumor=40%	Standard Treatment	~0		
DM Ping-Pong on Progression (cytostatic)				

Delta Tumor=5%	Standard Treatment	0.01	0.003-0.045	<0.001
Delta Tumor=10%	Standard Treatment	0.01	0.001-0.040	<0.001
Delta Tumor=20%	Standard Treatment	0.14	0.09-0.20	<0.001
Delta Tumor=40%	Standard Treatment	0.24	0.17-0.32	<0.001
FD Cocktail Dose-Skipping (cytostatic)				
Delta Tumor=5%	Standard Treatment	10.4	7.6-14.2	<0.001
Delta Tumor=10%	Standard Treatment	10.4	7.6-14.2	<0.001
Delta Tumor=20%	Standard Treatment	10.4	7.6-14.2	<0.001
Delta Tumor=40%	Standard Treatment	10.4	7.6-14.2	<0.001
FD Ping-Pong Dose-Skipping (cytostatic)				
Delta Tumor=5%	Standard Treatment	3.3	2.5-4.4	<0.001
Delta Tumor=10%	Standard Treatment	4.2	3.2-5.6	<0.001
Delta Tumor=20%	Standard Treatment	4.4	3.3-5.9	<0.001
Delta Tumor=40%	Standard Treatment	3.7	2.8-5.0	<0.001

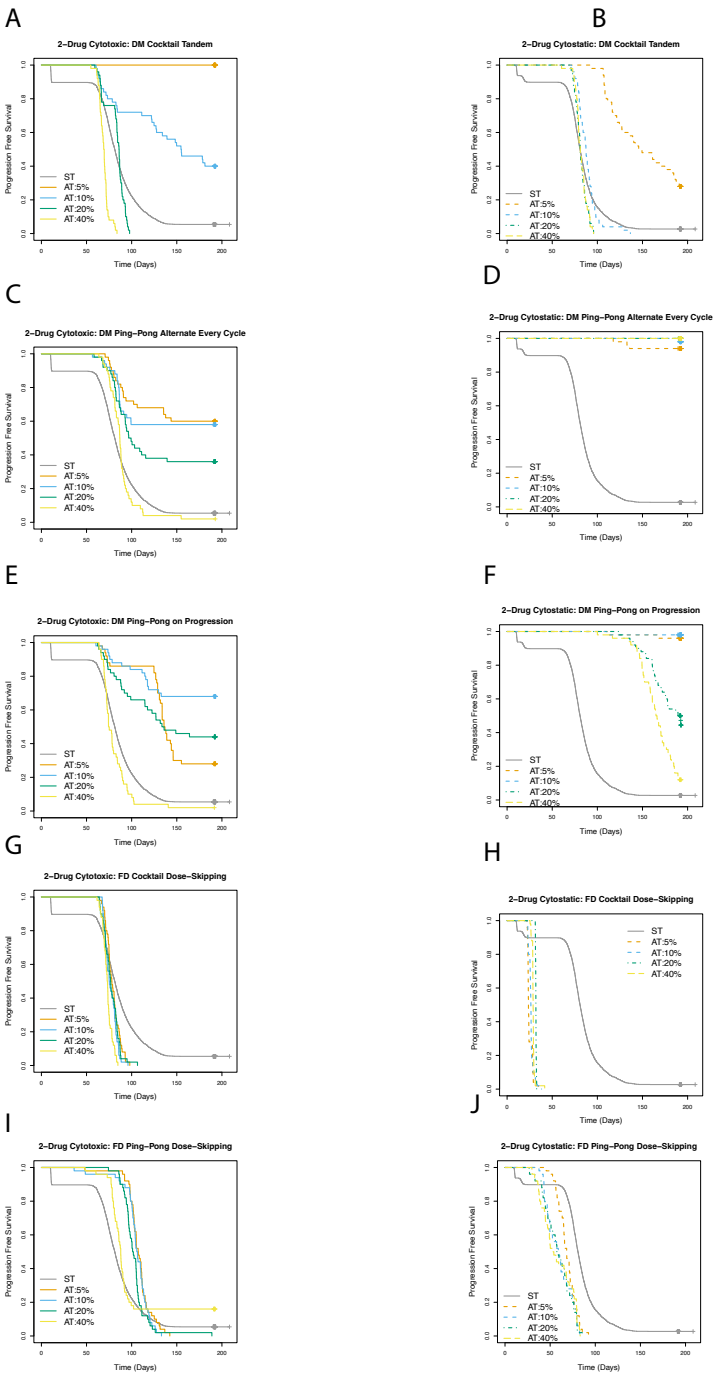

Figure 4.5. Effect of the delta tumor parameter on determining the outcome of adaptive therapy using two cytotoxic or two cytostatic drugs. Survival outcome comparing dose modulation treatment protocol with Delta Tumor=5%, 10%, 20%, or 40% using a single cytotoxic (Fig. 4.5A), or a single cytostatic drug (Fig. 4.5B) relative to standard

treatment. Survival outcome comparing dose-skipping treatment protocol with Delta Tumor=5%, 10%, 20%, or 40% using a single cytotoxic (Fig. 4.5C), or a single cytostatic drug (Fig. 4.5D).

How much to change the dose for the dose modulation protocols

For treatment using two cytotoxic drugs, all the dose modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, and DM Ping-Pong on Progression) work well when Delta Dose=25%, 50%, or 75%; with the exception of DM Cocktail Tandem with Delta Dose=25%. For treatment using two cytostatic drugs, the ping-pong protocols (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression) works well when delta dose=25%, 50%, or 75%. In general, the larger delta doses work best, for both cytotoxic and cytostatic drugs (Fig. 4.6), with the exception of DM Ping-Pong on Progression with cytotoxic drugs (Fig. 4.6E, Table 4.6).

Table 4.6: Effect of the delta dose parameter on determining the outcome of dose modulation adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
Delta Dose=25%	Standard Treatment	2.0	1.5-2.7	<0.001
Delta Dose=50%	Standard Treatment	0.29	0.20-0.41	<0.001
Delta Dose=75%	Standard Treatment	~0		
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
Delta Dose=25%	Standard Treatment	0.26	0.17-0.40	<0.001

Delta Dose=50%	Standard Treatment	0.26	0.17-0.40	<0.001
Delta Dose=75%	Standard Treatment	0.19	0.12-0.30	<0.001
DM Ping-Pong on Progression (cytotoxic)				
Delta Dose=25%	Standard Treatment	0.02	0.01-0.07	<0.001
Delta Dose=50%	Standard Treatment	0.11	0.06-0.19	<0.001
Delta Dose=75%	Standard Treatment	0.32	0.22-0.47	<0.001
DM Cocktail Tandem (cytostatic)				
Delta Dose=25%	Standard Treatment			Not Significant
Delta Dose=50%	Standard Treatment			Not Significant
Delta Dose=75%	Standard Treatment	0.69	0.51-0.92	<0.05
DM Ping-Pong Alternated Every Cycle (cytostatic)				
Delta Dose=25%	Standard Treatment	~0		
Delta Dose=50%	Standard Treatment	~0		
Delta Dose=75%	Standard Treatment	~0		
DM Ping-Pong on Progression (cytostatic)				
Delta Dose=25%	Standard Treatment	0.03	0.01-0.07	<0.001
Delta Dose=50%	Standard Treatment	~0		

Delta Dose=75%	Standard Treatment	0.03	0.01-0.07	<0.001

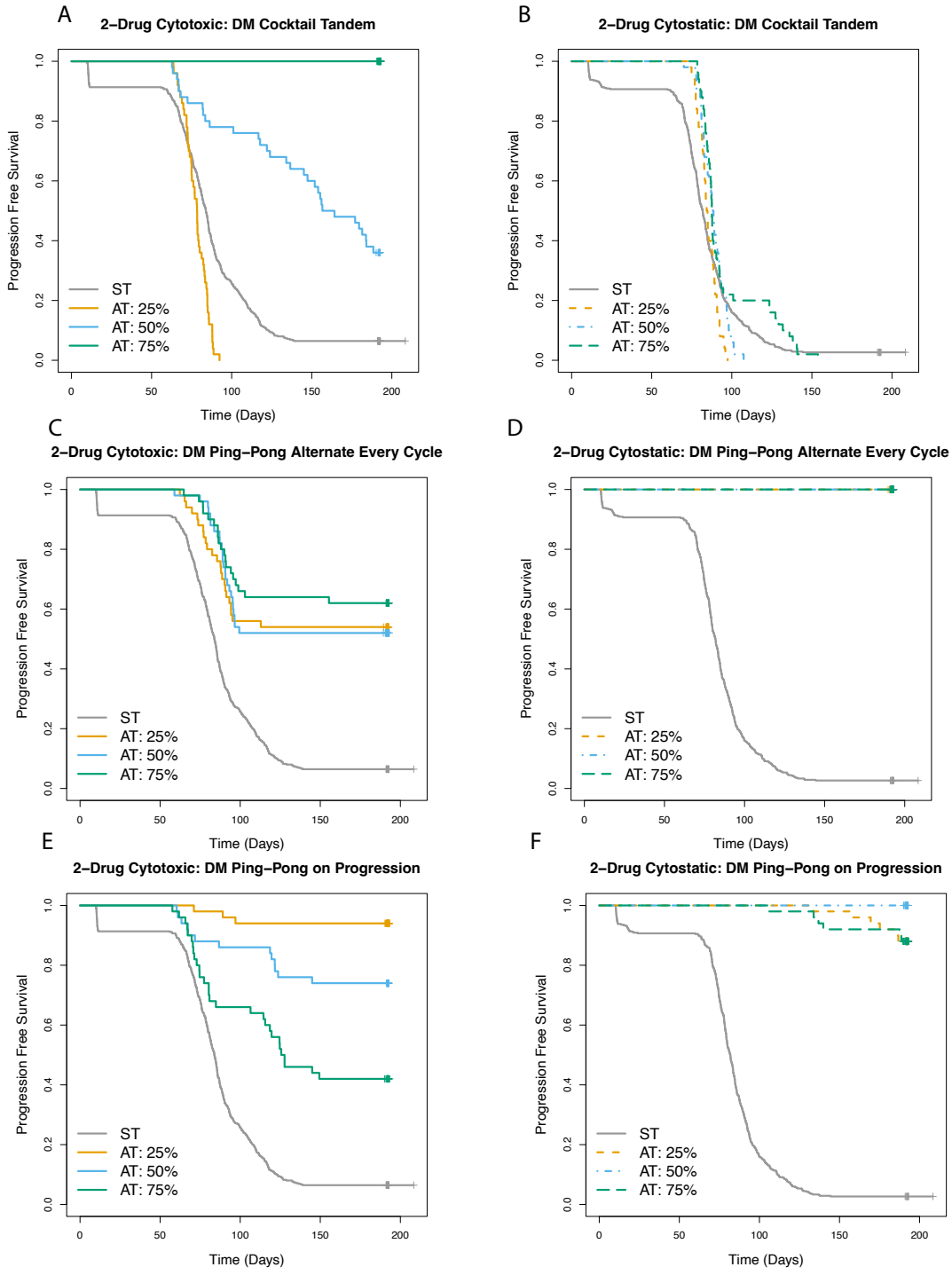

Figure 4.6. Effect of the delta dose parameter on determining the outcome of dose modulation adaptive therapy using two cytotoxic or two cytostatic drugs. Survival outcome for treatment as per the dose modulation protocol with Delta Dose=25%, 50%,

or 75% relative to standard treatment using a single cytotoxic drug (Fig. 4.6A), or a single cytostatic drug (Fig. 4.6B).

Effects of pausing treatment when tumor burden falls below a certain level

For treatment using two cytotoxic or two cytostatic drugs, the dose-modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression) work best when treatment is paused sooner than later when the tumor is responding, that is, triggering a treatment vacation when the tumor shrinks by 20%, for example, works better than waiting for the tumor to shrink by 50%, or 90% (Fig. 4.7, Table 4.7). The only exception is DM Ping-Pong on Progression (Fig. 4.7E), where pausing treatment at 50% is working better than 20%. A similar effect is observed for treatment using two cytotoxic (Fig. 4.7G, Fig 4.7I, Table 4.7) or two cytostatic drugs (Fig. 4.7H, 4.7J, Table 4.7) as per the intermittent protocol, where using a lower threshold for pausing treatment works better, however, the effect size is not strong for treatment using two cytotoxic drugs. In general, stopping treatment sooner than later, when the tumor is responding works well, for both treatment using two cytotoxic or two cytostatic drugs.

Table 4.7: Effect of stopping treatment when tumor burden falls below a certain level for treatment using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	0.16	0.11-0.25	<0.001

Treatment vacation when tumor shrinks by 50%	Standard Treatment	0.28	0.20-0.40	<0.001
Treatment vacation when tumor shrinks by 90%	Standard treatment	3.4	2.5-4.5	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	0.12	0.07-0.20	<0.001
Treatment vacation when tumor shrinks by 50%	Standard Treatment	0.21	0.14-0.32	<0.001
Treatment vacation when tumor shrinks by 90%	Standard treatment	0.50	0.35-0.70	<0.001
DM Ping-Pong on Progression (cytotoxic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	0.31	0.22-0.44	<0.001
Treatment vacation when tumor shrinks by 50%	Standard Treatment	0.13	0.08-0.22	<0.001
Treatment vacation when tumor shrinks by 90%	Standard treatment			Not Significant
FD Cocktail Intermittent (cytotoxic)				

Stop when shrinks by 5%	Standard Treatment	1.4	1.0-1.9	<0.05
Stop when shrinks by 10%	Standard Treatment			Not Significant
Stop when shrinks by 20%	Standard Treatment	1.6	1.2-2.1	<0.01
Stop when shrinks by 50%	Standard Treatment	1.6	1.2-2.2	<0.001
FD Ping-Pong Intermittent (cytotoxic)				
Stop when shrinks by 5%	Standard Treatment	1.4	1.1-1.9	<0.05
Stop when shrinks by 10%	Standard Treatment	1.6	1.2-2.2	<0.01
Stop when shrinks by 20%	Standard Treatment	1.6	1.2-2.2	<0.01
Stop when shrinks by 50%	Standard Treatment	1.8	1.3-2.4	<0.001
DM Cocktail Tandem (cytostatic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	0.73	0.55-0.97	<0.05
Treatment vacation when tumor shrinks by 50%	Standard Treatment			Not Significant
Treatment vacation when	Standard treatment			Not Significant

tumor shrinks by 90%				
DM Ping-Pong Alternated Every Cycle (cytostatic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	~0		
Treatment vacation when tumor shrinks by 50%	Standard Treatment	~0		
Treatment vacation when tumor shrinks by 90%	Standard treatment	~0		
DM Ping-Pong on Progression (cytostatic)				
Treatment vacation when tumor shrinks by 20%	Standard Treatment	~0		
Treatment vacation when tumor shrinks by 50%	Standard Treatment	~0		
Treatment vacation when tumor shrinks by 90%	Standard treatment	~0		
FD Cocktail Intermittent (cytostatic)				
Stop when shrinks by 5%	Standard Treatment	0.70	0.52-0.93	<0.05
Stop when shrinks by 10%	Standard Treatment			Not Significant

Stop when shrinks by 20%	Standard Treatment	0.72	0.54-0.96	<0.05
Stop when shrinks by 50%	Standard Treatment	1.3	1.0-1.8	<0.05
FD Ping-Pong Intermittent (cytostatic)				
Stop when shrinks by 5%	Standard Treatment	~0		
Stop when shrinks by 10%	Standard Treatment	~0		
Stop when shrinks by 20%	Standard Treatment	~0		
Stop when shrinks by 50%	Standard Treatment	~0		

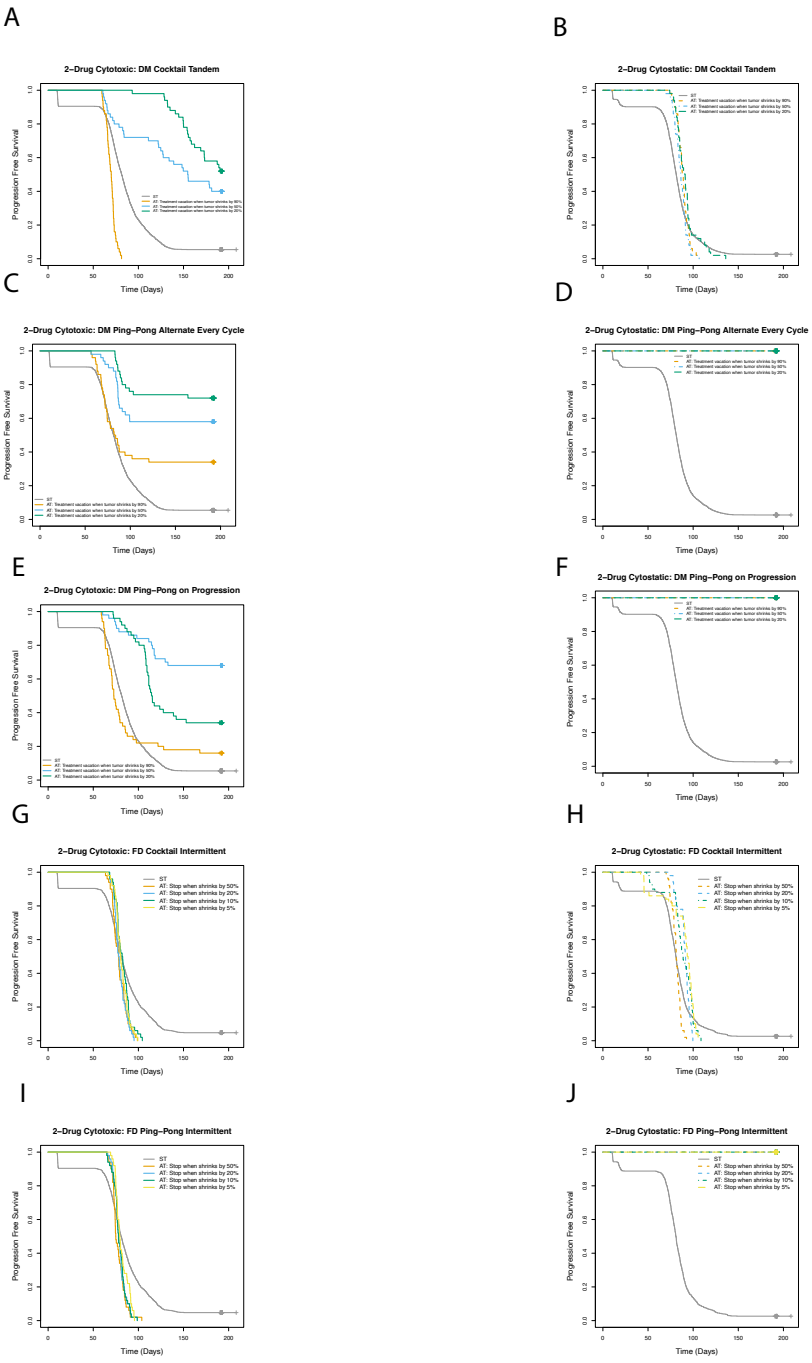

Figure 4.7. Effect of stopping treatment when tumor burden falls below a certain level for treatment using two cytotoxic or two cytostatic drugs. For treatment as per the intermittent protocol using either a single cytotoxic (Fig. 4.7A), or a single cytostatic drug (Fig. 4.7B), the threshold for stopping treatment was varied as the tumor shrinks by

5%, 10%, 20%, or 50% of the pre-treatment baseline. Survival outcome for treatment using a single cytotoxic drug (Fig. 4.7C), or a single cytostatic drug (Fig. 4.7D) as the trigger for treatment vacation is when the tumor shrinks by 20%, 50%, or 90%.

Drug dosage level at which adaptive therapy is initiated and capped

Standard treatment for cytotoxic drugs begins to work at 25%of MTD as we lower the drug dosages, and works perfectly for 15% MTD, and at 10% begins to lose control of some of the tumors (Fig. 4.8G); for FD Cocktail Intermittent, 10% of MTD does not work but 15% and 25% work perfectly, and at 50% of MTD and above it does not work (Fig. 4.8G). Goldilocks effect in most of these cases is observed, where too much drug selects for resistance or too less drug is unable to control the tumor. At low extremes of the drug, the tumor grows out control, and at high extremes of the drug, the resistant clones are taking over the tumor. For cytostatic drugs, standard treatment at 25% or 50% of MTD works well (Fig. 4.8F), while standard treatment at 10%, 15%, 75%, and 100% performs poorly (Fig. 4.8F); for FD Cocktail Intermittent, treatment at 50% of MTD results in perfect survival, while survival outcome worsens with treatment at 10%, 15%, 25%, 75%, or 100% of MTD (Fig. 4.8H), exhibiting a Goldilocks effect for both standard treatment as well as adaptive therapy in many of these cases.

Table 4.8: Effect of administering treatment at a range of different drug dosages for adaptive therapy using two cytotoxic or two cytostatic drugs

Experimental Condition	**Comparison Condition**	**Hazard Ratio**	**95% CI**	***p*-value**
DM Cocktail Tandem (cytotoxic)				
25% MTD	Standard Treatment at 25% MTD	~0		

50% MTD	Standard Treatment at 50% MTD	0.08	0.05-0.12	<0.001
75% MTD	Standard Treatment at 75% MTD	0.04	0.02-0.08	<0.001
100% MTD	Standard treatment at 100% MTD	0.24	0.16-0.35	<0.001
DM Ping-Pong Alternated Every Cycle (cytotoxic)				
25% MTD	Standard Treatment at 25% MTD	~0		
50% MTD	Standard Treatment at 50% MTD	~0		
75% MTD	Standard Treatment at 75% MTD	0.24	0.16-0.37	<0.001
100% MTD	Standard treatment at 100% MTD	0.20	0.13-0.32	<0.001
DM Ping-Pong on Progression (cytotoxic)				
25% MTD	Standard Treatment at 25% MTD	~0		
50% MTD	Standard Treatment at 50% MTD	0.004	0.001-0.031	<0.001
75% MTD	Standard Treatment at 75% MTD	0.07	0.04-0.13	<0.001

100% MTD	Standard treatment at 100% MTD	0.12	0.07-0.21	<0.001
FD Cocktail Dose-Skipping (cytotoxic)				
10% MTD	Standard Treatment at 10% MTD	6.3	4.3-9.4	<0.001
Standard Treatment at 15% MTD	15% MTD	~0		
Standard Treatment at 25% MTD	25% MTD	~0		
50% MTD	Standard treatment at 50% MTD	27.1	14.7-50.0	<0.001
75% MTD	Standard treatment at 75% MTD			Not Significant
100% MTD	Standard treatment at 100% MTD	1.7	1.2-2.3	<0.01
FD Ping-Pong Dose-Skipping (cytotoxic)				
10% MTD	Standard Treatment at 10% MTD	9.2	6.1-14.0	<0.001
Standard Treatment at 15% MTD	15% MTD	~0		
Standard Treatment at 25% MTD	25% MTD	~0		
50% MTD	Standard treatment at 50% MTD	27.1	14.7-50.0	<0.001

75% MTD	Standard treatment at 75% MTD	0.45	0.33-0.62	<0.001
100% MTD	Standard treatment at 100% MTD	1.9	1.4-2.6	<0.001
FD Cocktail Intermittent (cytotoxic)				
10% MTD	Standard Treatment at 10% MTD	2.6	1.9-3.7	<0.001
15% MTD	Standard Treatment at 15% MTD			Not Significant
25% MTD	Standard Treatment at 25% MTD	~0		
50% MTD	Standard treatment at 50% MTD	1.5	1.1-2.1	<0.05
75% MTD	Standard treatment at 75% MTD	3.8	2.7-5.5	<0.001
100% MTD	Standard treatment at 100% MTD	1.9	1.4-2.7	<0.001
FD Ping-Pong Intermittent (cytotoxic)				
10% MTD	Standard Treatment at 10% MTD	8.8	5.9-13.0	<0.001
Standard treatment at 15% MTD	15% MTD	~0		
25% MTD	Standard Treatment at 25% MTD	~0		

50% MTD	Standard treatment at 50% MTD			Not Significant
75% MTD	Standard treatment at 75% MTD	2.6	1.9-3.7	<0.001
100% MTD	Standard treatment at 100% MTD	2.0	1.5-2.9	<0.001
DM Cocktail Tandem (cytostatic)				
25% MTD	Standard Treatment at 25% MTD	4.7	3.3-6.7	<0.001
50% MTD	Standard Treatment at 50% MTD	~0		
75% MTD	Standard Treatment at 75% MTD			Not Significant
100% MTD	Standard treatment at 100% MTD			Not Significant
DM Ping-Pong Alternate Every Cycle (cytostatic)				
25% MTD	Standard Treatment at 25% MTD	17.1	11.8-24.8	<0.001
50% MTD	Standard Treatment at 50% MTD	~0		
75% MTD	Standard Treatment at 75% MTD	~0		

100% MTD	Standard treatment at 100% MTD	~0		
DM Ping-Pong on Progression (cytostatic)				
25% MTD	Standard Treatment at 25% MTD	14.4	10.0-20.8	<0.001
50% MTD	Standard Treatment at 50% MTD	0.27	0.16-0.45	<0.001
75% MTD	Standard Treatment at 75% MTD	~0		
100% MTD	Standard treatment at 100% MTD	0.01	0.001-0.043	<0.001
FD Cocktail Dose-Skipping (cytostatic)				
10% MTD	Standard Treatment at 10% MTD	2.3	1.7-3.2	<0.001
15% MTD	Standard Treatment at 15% MTD	2.4	1.7-3.3	<0.001
25% MTD	Standard Treatment at 25% MTD	3.1	2.2-4.5	<0.001
50% MTD	Standard treatment at 50% MTD	123.7	49.6-308.3	<0.001
75% MTD	Standard treatment at 75% MTD	29.7	15.8-55.7	<0.001
100% MTD	Standard treatment at 100% MTD	19.5	11.1-34.3	<0.001

FD Ping-Pong Dose-Skipping (cytostatic)				
10% MTD	Standard Treatment at 10% MTD	3.5	2.5-4.9	<0.001
15% MTD	Standard Treatment at 15% MTD	6.0	4.2-8.5	<0.001
25% MTD	Standard Treatment at 25% MTD	10.7	7.4-15.6	<0.001
Standard Treatment at 50% MTD	50% MTD	~0		
75% MTD	Standard treatment at 75% MTD	7.1	4.8-10.4	<0.001
100% MTD	Standard treatment at 100% MTD	19.5	11.1-34.3	<0.001
FD Cocktail Intermittent (cytostatic)				
10% MTD	Standard Treatment at 10% MTD	1.9	1.4-2.6	<0.001
15% MTD	Standard Treatment at 15% MTD	3.7	2.6-5.3	<0.001
25% MTD	Standard Treatment at 25% MTD	2.9	2.0-4.1	<0.001
50% MTD	Standard treatment at 50% MTD	~0		
75% MTD	Standard treatment at 75% MTD			Not Significant

100% MTD	Standard treatment at 100% MTD			Not Significant
FD Ping-Pong Intermittent (cytostatic)				
10% MTD	Standard Treatment at 10% MTD	4.3	3.1-6.0	<0.001
15% MTD	Standard Treatment at 15% MTD	5.8	4.0-8.3	<0.001
25% MTD	Standard Treatment at 25% MTD	11.2	7.6-16.5	<0.001
50% MTD	Standard treatment at 50% MTD	~0		
75% MTD	Standard treatment at 75% MTD	~0		
100% MTD	Standard treatment at 100% MTD	~0		

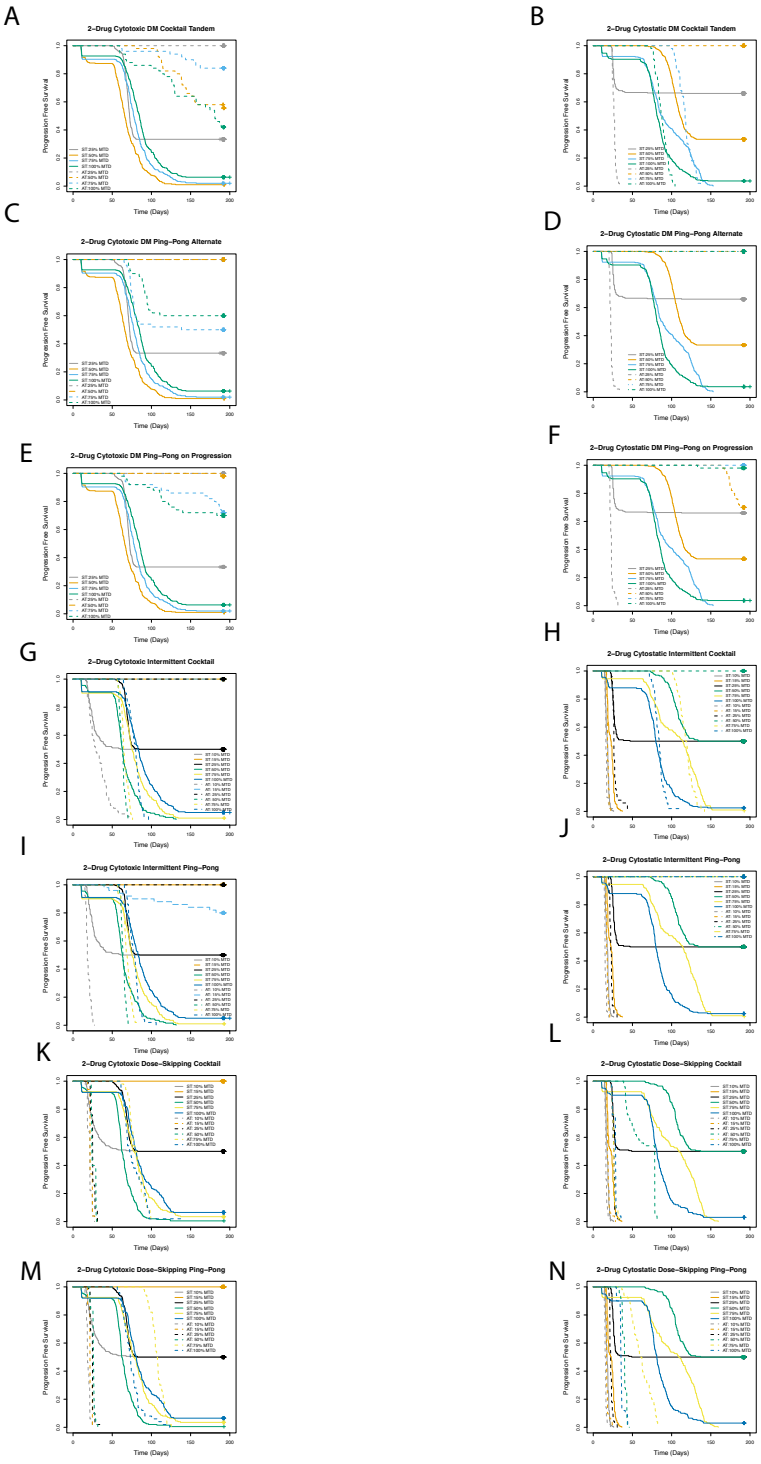

Figure 4.8. Effect of administering treatment at a range of different drug dosages for adaptive therapy using two cytotoxic or two cytostatic drugs. Survival outcome comparing treatment as per the dose modulation protocol relative to ST, starting and capping dosing at 25%, 50%, 75%, or 100% of MTD, for treatment using a single

cytotoxic drug (Fig. 4.8A), or a single cytostatic drug (Fig. 4.8B). Survival outcome for treatment as per dose-skipping protocol administered at 35%, 50%, 75%, or 100% of MTD relative to standard treatment using either a single cytotoxic drug (Fig. 4.8C), or a single cytostatic drug (Fig. 4.8D). Survival outcome for treatment as per the intermittent protocol administered at 10%, 15%, 25%, 50%, 75%, or 100% of MTD relative to ST for treatment using a single cytotoxic (Fig. 4.8E), or a single cytostatic (Fig. 4.8F) drug.

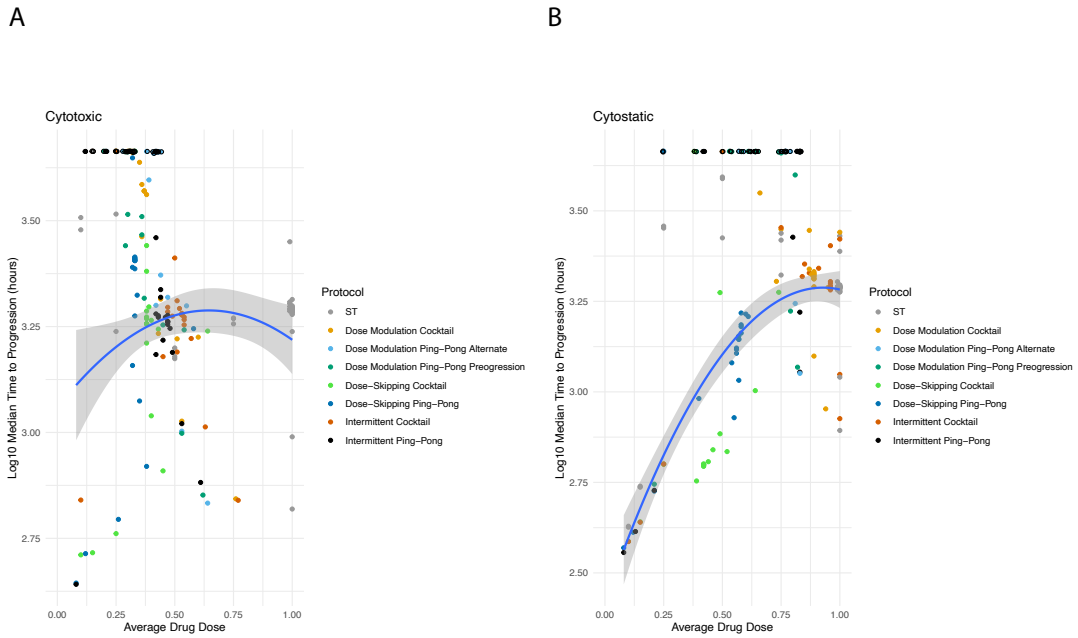

Figure 4.9. Summarizing the relationship between drug dose and time to progression for adaptive therapy using two cytotoxic or two cytostatic drugs. In each panel, the average amount of drug used per timestep between the start of therapy and the time of progression is plotted on the X-axis, and the median time to progression for that protocol under those parameter values are plotted on the Y-axis, for treatment using either a single cytotoxic drug (A) or a single cytostatic drug (B). The points are colored based on the specific protocol. Open circles indicate data points that are censored as less than 50% of test-subjects have progressed, and are not included in the calculation. A quadratic fit to the curve along with the confidence intervals have been indicated in the figure panels. Each point represents a specific Kaplan-Meier survival curve for a given set of parameter values.

Median TTP versus Average Drug Dose

I plotted log10 median TTP versus average drug dose including all data points except for which a median TTP is not available (since less than 50% of the test subjects

has progressed) and fitted the curve to a quadratic plot. For treatment using two cytotoxic drugs (Fig. 9A), median R-squared value is 0.03479 and adjusted R-squared is 0.0206. For treatment using two cytostatic drugs (Fig. 10B), median R-squared value is 0.6165 and adjusted R-squared value is 0.6105.

Discussion

Designing adaptive therapy protocols using two drugs is challenging as the number of parameters increase exponentially with each additional drug. Furthermore, we seek to investigate how the mechanism of action of the drugs, that is, whether or not the drugs are cytotoxic or cytostatic impacts survival outcome. Thus, we have investigated seven different treatment protocols, the dose modulation (DM) protocols: DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression; and the fixed-dose (FD) protocols: FD Cocktail Dose-Skipping, FD Ping-Pong Dose-Skipping, FD Cocktail Intermittent, FD Ping-Pong Intermittent; for treatment using either two cytotoxic or two cytostatic drugs.

We observed, for treatment using two cytotoxic drugs, all dose modulation protocols, namely, DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, and DM Ping-Pong on Progression, as well the fixed-dose protocol FD Ping-Pong Dose-Skipping work well, increasing TTP relative to standard treatment. For treatment using two cytostatic drugs, the ping-pong protocols, namely, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, and FD Ping-Pong Intermittent work well, improving survival outcome relative to standard treatment.

Our results show that fitness cost, as manifested in increased doubling time for the drug-resistant cells in the absence of the drug, is required for adaptive therapy to work for

both treatments using two cytotoxic or two cytostatic drugs. In general, a higher fitness cost leads to improved survival outcome, increasing TTP relative to lower fitness costs. However, a caveat with very high fitness cost is that the tumor burden is also increasing at a faster rate, which could translate to hitting the carrying capacity, and this effect might be especially pronounced under low amounts of the drugs. We observed FD Dose-Skipping treatment protocols working poorly under high fitness costs.

Cell replacement is an important parameter in our model as it is a parameter that governs whether or not a dividing cell would be allowed to replace a neighbor when there is no space available in the Moore neighborhood and, as such it is a measure of cell competition in the tumor. Our results indicate adaptive therapy works best at higher replacement rates and, in general, works best under conditions of 100% replacement. Furthermore, we observe this effect for both cytotoxic as well as cytostatic drugs. This suggests that adaptive therapy might work especially well when there is strong competition among the cell types constituting the tumor.

We observe, in general, adaptive therapy using two cytotoxic drugs works best under conditions of high turnover. However, in general, treatment using two cytostatic drugs seems to work best when the cell turnover is low.

We find, in general, adaptive therapy works best when drug doses are adjusted as soon as a change in tumor burden is detected, for treatment using either two cytotoxic or two cytostatic drugs. Furthermore, in general, the survival outcome worsens as Delta Tumor is progressively increased, with the best survival outcome with Delta Tumor=5% and the worst with Delta Tumor=40%. These results are in agreement.

We find, in general, for treatment using two cytotoxic or two cytostatic drugs, that it is best to pause treatment sooner than later when the tumor is shrinking. As such, for treatment as per the dose modulation protocol, triggering a treatment vacation when the tumor has shrunk by 20% works better than triggering a treatment vacation when the tumor has shrunk by 50%, which in turn works better than triggering a treatment vacation when the tumor has shrunk by 90%. We observe the same trend for treatment as per the intermittent protocol. As such, choosing to pause treatment when the tumor shrinks by 5% works better than choosing to pause treatment when the tumor shrinks by 10%, which is works better than choosing to pause treatment when the tumor shrinks by 20%, which work better than choosing to pause treatment when the tumor shrinks by 50%.

We observe, in general, for both treatment using either two cytotoxic or two cytostatic drugs, that too low dosage of a drug has poor survival outcome, and too high dosage of a drug also has poor survival outcome, with intermediate level of drug dosages having the best survival outcome. Thus, a Goldlilocks level of drug dosing works best for both treatments using either two cytotoxic or two cytostatic drugs. Consistent with this observation, fitting median TTP versus average drug dose to a quadratic results in significant fit.

There are several limitations to this work. We have not modeled tissue architecture or normal cells inside the tumors. Modeling blood vasculature and capillary architecture could affect treatment outcomes in important ways. In the future, it would be interesting to explore how these adaptive therapy protocols would perform in a 3-dimensional tumor model perfused with blood vessels and capillaries.

Conclusions

While the dose modulation protocols (DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression), as well as the fixed-dose protocol (FD Ping-Pong Dose-Skipping) works well for treatment using two cytotoxic drugs, the ping-pong protocols except dose-skipping (DM Ping-Pong Alternate Every Cycle, DM Ping-Pong on Progression, and FD Ping-Pong Intermittent) works well for treatment using two cytostatic drugs, increasing TTP relative to standard treatment at maximum tolerated dose. In general, we observe adaptive therapy protocols work best under conditions of higher fitness cost, increased replacement rates, and higher turnover, with the exception of cytostatic drugs generally working better under conditions of low turnover. For the dose modulation protocols, as well as fixed-dose protocols, adaptive therapy works best when the doses are adjusted as soon as a change in tumor burden is detected. For the dose modulation protocols, in general, a relatively higher value for the delta dose parameter works best. We find that it is best to pause treatment sooner than later, when the tumor is shrinking. As such, triggering a treatment vacation or choosing to pause treatment when the tumor has shrunk by a relatively lower percentage relative to the baseline tumor burden at which therapy was initiated, works better than waiting for the tumor to shrink by a relatively higher percentage relative to that baseline. In general, for treatment using either two cytotoxic or two cytostatic drugs, an intermediate level of drug dosage works best, and worse survival outcome is observed with too little or too much drug, in accordance with the Goldilocks' principle. Our results suggest adaptive therapy can be considerably improved by developing accurate and sensitive measurements of tumor burden, as well as by assaying for the level of cell turnover inside the tumor. Furthermore, our results lend support to the idea that it is indeed possible to

transform cancer from an acute and lethal disease, which results in death, to a chronic disease, which does not lead to death.

CHAPTER 5

COMPARISON OF IN SILICO MODELING RESULTS TO PRECLINICAL MICE EXPERIMENTS

Abstract

Computational models that accurately predict clinical outcomes are an important toolkit for translational research, having the potential to transform the field of medical oncology. However, calibrating computational models to predict experimental outcomes can be a challenging task, especially when the model involves a large number of parameters. In this article our goal was to match the results of the preclinical mice experiments conducted in mice with breast cancer for treatment using either a single cytotoxic drug or two cytotoxic drugs. Our results indicate it is possible to predict the treatment outcome using the hybrid agent-based model that we have developed to simulate a wide variety of adaptive therapy treatment protocols. Specifically, we were able to match the order in which the various treatment protocols performed best-to-worst, for both treatment using a single cytotoxic drug, or two cytotoxic drugs.

Introduction

Calibrating hybrid agent-based models to a specific experimental system is an important step in translational research, as it enables the computational model to predict the outcome of the experimental system. In chapters 2, 3 (cite the article here), and 4, I have developed and implemented hybrid agent-based models to simulate adaptive therapy using a single, or two drugs. In this chapter, the goal was to match the simulation results to the experimental preclinical experiments conducted in mice with breast cancer. For the

individual adaptive therapy protocols, I investigated the ranges of the relevant parameter space in the model, for treatment using a single cytotoxic drug, or two cytotoxic drugs, in order to match the rank order of the experimental results from best-to-worst progression-free survival outcomes.

Materials and Methods

For treatment using a single cytotoxic drug, please see Chapter 2, Materials and Methods section for details of the model. For treatment using two cytotoxic drugs, please see Chapter 4, Materials and Methods for details of the model. For treatment using a single cytotoxic drug, the adaptive therapy protocols of interest were dose modulation and intermittent. For treatment using two cytotoxic drugs, the adaptive therapy protocols of interest were DM Cocktail Tandem, DM Ping-Pong Alternate Every Cycle, FD Cocktail Intermittent, and FD Ping-Pong Intermittent. For each of these protocols, I explored the parameter space for the relevant parameters of the model, in a heuristic manner, in order to match the preclinical mice data. The parameter values that were used to run the model for treatment using a single cytotoxic drug are indicated in Table 5.1, and that were used to run the model for treatment using two cytotoxic drugs are indicated in Table 5.2.

The resistant cells (singly resistant for treatment using a single drug, or doubly resistant cells for treatment using two drugs) both have a division rate of 0.02 per hour. The sensitive cells (for treatment using a single drug) have a division rate of 0.06 per hour and the doubly sensitive cells (for treatment using two drugs) have a division rate of 0.10 per hour. MTD dosage value for treatment using a single drug was set to 2.5 units, and MTD dosage value for treatment using two cytotoxic drugs was set to 2.5 units for

each of the two drugs, thus the total dosage equaled to 5 units for a cocktail application, and 2.5 units of the specific drug for the ping-pong protocols. We do not know the true division rates, or the true MTDs, nor do we know the true replacement probability inside a tumor. So, we varied those parameters to see if it would match the experimental results.

We have made some modifications to our criterion for progression from (Thomas et al. 2022). The modified survival criterion is as follows: If the tumor burden equaled or exceeded 99% of the carrying capacity at any point after initiation of therapy, or the rolling average of the total number of resistant cells over 500 time-steps equaled or exceeded 50% of the carrying capacity, then the particular run is scored as "Progressed" and the time at which progression takes place after therapy initiation is noted.

Table 5.1: Parameter values for matching the results of the mice experiments using a single cytotoxic drug. The italics indicate changes in parameter values relative to Chapter 2 that were necessary to match the experimental results.

Parameter	**Value**
Cell division rate: sensitive	0.06 per hour
Cell division rate: resistant	0.02 per hour
Background death rate	0.01 per hour
Replacement probability	*1.0*
Delta Tumor	10%
Delta Dose	50%
Probability of death due to drug potency (Ψ)	0.04 per unit drug concentration
Maximum tolerated dose (MTD)	2.5 units
Minimum drug dose	0.5 units
Drug on time	1 hour
Frequency of drug application	Once every 24 hour
Check tumor burden	Every 3 days
Drug decay	10% per hour

Drug diffusion rate	2.0
Tumor size triggering treatment	Tumor burden is 50% or more of the carrying capacity
Mutation rate	1e-3 per cell division
Measurement noise standard deviation (SD)	5 cells
Total grid size	100 by 100
Duration of simulation	5000 hour
Stop dosing/initiate treatment vacation when (DM protocols only):	Tumor burden is less than or equal to 25% of carrying capacity
Doubling time of sensitive cells	13.86 hour
Doubling time of resistant cells	69.3 hour

Table 5.2: Parameter values for matching the results of the mice experiments using two cytotoxic drugs. The italics indicate changes in parameter values relative to Chapter 3 and Chapter 4 that were necessary to match the experimental results.

Parameter	**Value**
Cell division rate: doubly sensitive	*0.10 per hour*
Cell division rate: singly resistant	*0.06 per hour*
Cell division rate: doubly resistant	0.02 per hour
Background death rate	0.01 per hour
Replacement probability	*1.0*
Delta Tumor	10%
Delta Dose	50%
Probability of death due to drug potency (Ψ)	0.04 per unit drug concentration
Maximum tolerated dose (MTD): Drug 1	2.5 units
Maximum tolerated dose (MTD): Drug 2	2.5 units
Minimum drug dose	0.5 units
Drug on time	1 hour
Frequency of drug application	Once every 24 hour
Check tumor burden	Every 3 days
Drug decay	10% per hour
Drug diffusion rate	2.0

Tumor size triggering treatment	Tumor burden is 50% or more of the carrying capacity
Mutation rate	1e-3 per cell division
Measurement noise standard deviation (SD)	5 cells
Total grid size	100 by 100
Duration of simulation	5000 hour
Stop dosing/initiate treatment vacation when (DM protocols only):	Tumor burden is less than or equal to 25% of carrying capacity
Doubling time of doubly sensitive cells	7.7 hour
Doubling time of doubly resistant cells	69.3 hour
Doubling time of singly resistant cells	13.86 hour

Results

The mouse experiments (experimental data produced by Sareh Seyedi) showed that the adaptive therapy protocols could be arranged in a specific rank order from best-to-worst progression-free survival outcome, both for treatment using a single cytotoxic drug, or two cytotoxic drugs. These experiments were carried out using MCF7 breast cancer cell lines that were selected to be resistant to the anti-cancer drugs fulvestrant and palbociclib for hormone refractory estrogen receptor (ER) positive breast cancer. For these mice experiments, the anti-cancer drug capecitabine was used for adaptive therapy using a single drug, and for adaptive therapy using two drugs, the two anti-cancer drugs used were capecitabine and gemcitabine. Because both of these drugs, gemcitabine and capecitabine work by killing cells, these drugs can be assumed to have a cytotoxic mechanism of action.

Matching simulation results to preclinical mice experiments for treatment using a single cytotoxic drug

For adaptive therapy using a single drug (capecitabine), the rank order from best to worst for progression-free survival outcomes are as follows:

1. Dose modulation
2. No treatment
3. Standard treatment and Intermittent were essentially equivalent

The computational simulation results (Fig. 5.1) are in agreement with the experimental data.

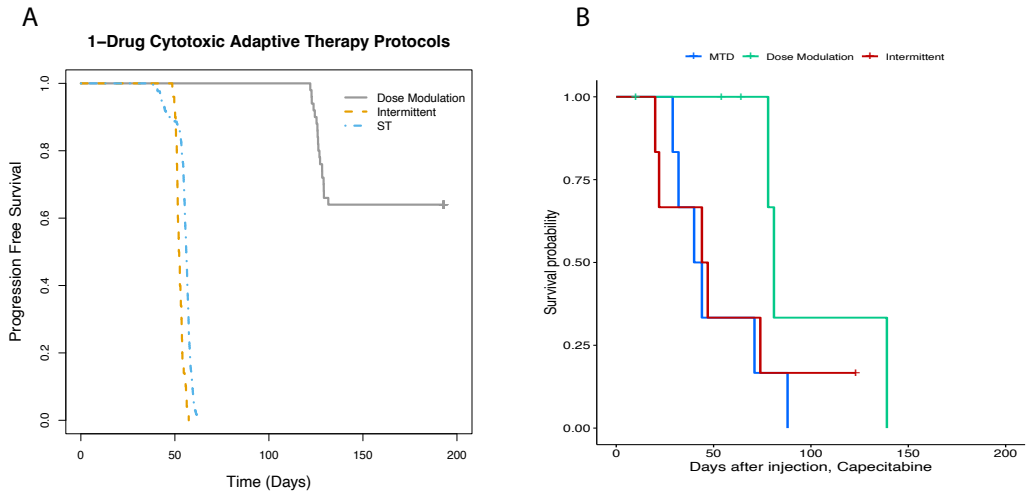

Figure 5.1: Comparison of simulation results to preclinical mice data for adaptive therapy using a single cytotoxic drug. Computational simulations of adaptive therapy using a single cytotoxic drug (Fig. 5.1A) and results of treating mice with breast cancer as per single-drug adaptive therapy protocols using the drug capecitabine (Fig 5.1B). For Fig 5.1B, the experimental data as well as the figure itself was generated by Sareh Seyedi.

Matching simulation results to preclinical mice experiments for treatment using two cytotoxic drugs

For adaptive therapy using two drugs (capecitabine and gemcitabine), the rank order from best to worst for progression free survival outcomes are as follows:

1. There is essentially a tie between DM Ping-Pong Alternate Every Cycle, FD Ping-Pong Intermittent, and DM Cocktail Tandem. All these protocols were essentially equivalent
2. No treatment
3. FD Cocktail Intermittent
4. Standard treatment using a cocktail drug formulation for the two drugs

The computational simulation results (Fig. 5.2) are in agreement with the experimental data.

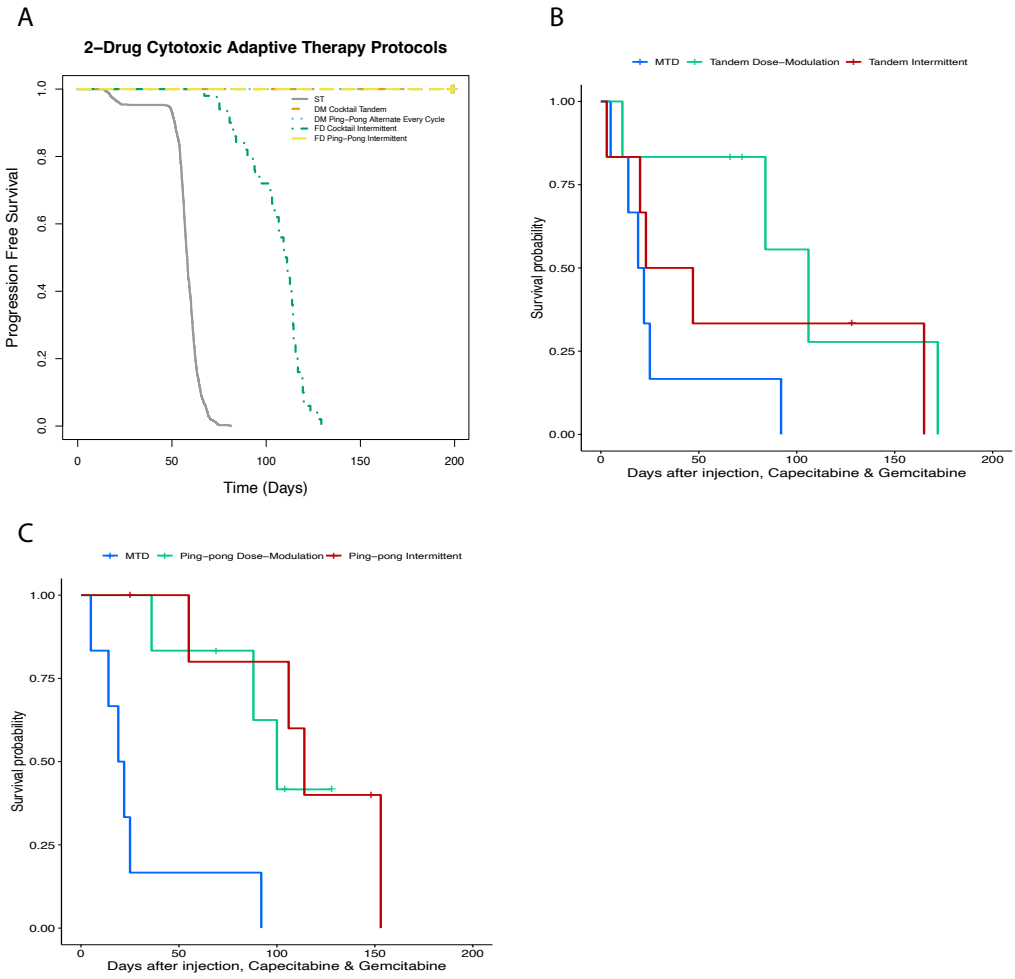

Figure 5.2: Comparison of simulation results to preclinical mice data for adaptive therapy using two cytotoxic drugs. Computational simulations of adaptive therapy using two cytotoxic drugs (Fig. 5.2A). Results of treating mice with breast cancer as per adaptive therapy protocols using the drug gemcitabine and capecitabine using a cocktail formulation of the drugs such that both the drugs are administered at the same time, referred to here as 'Tandem' (Fig. 5.2B), or rotating the drugs every treatment cycle, such that only one drug is applied at a time, referred to here as 'Ping-pong' (Fig. 5.2C) . For Fig 5.2B and Fig. 5.2C, the experimental data as well as the figures themselves were generated by Sareh Seyedi.

Discussion

The experimental results support the modeling results. The computational simulations resulted in a match for the rank order from best-to-worst for progression-free

survival outcome, both for treatment using either a single drug, or two drugs. However, given the number of parameters in the system, there is the potential of overfitting the experimental data.

CHAPTER 6

CONCLUSION

Chapter 1 gives an introduction to adaptive therapy. Chapter 2 investigates adaptive therapy using a single cytotoxic or a single cytostatic drug. Chapter 3 includes a publication involving investigations of multi-drug adaptive therapy protocols using 2 drugs. Chapter 4 investigates adaptive therapy using two cytotoxic or two cytostatic drugs. Chapter 5 involves matching computational simulation results using the developed hybrid agent-based model to preclinical mice data for both treatment using a single cytotoxic drug or two cytotoxic drugs. Chapter 6 discusses the conclusion.

Our results show adaptive therapy significantly improves survival outcome and increases TTP relative to standard treatment at maximum tolerated dose, under a wide variety of conditions, for treatment using either a single cytotoxic, a single cytostatic, two cytotoxic, or two cytostatic drugs. We have developed three adaptive therapy protocols for single drug adaptive therapy, and seven adaptive therapy protocols for treatment using two drugs. These protocols work for treatment using cytotoxic or cytostatic drugs. An interesting avenue for future research would be to be able to combine drugs with different mechanism of actions in a single treatment cycle, and developing treatment schedules that combines a single or multiple drugs. Possible avenues for future research would be to investigate adaptive therapy using 3 or more drugs, possibly utilizing optimal control and other machine learning approaches. Because adaptive therapy has so much potential to transform personalized medicine, it would be interesting to use patient-specific data to calibrate the models and develop personalized regimens for the particular cancer patient.

Extending the model to 3-dimesnisons, simulating both normal and cancer cells, as well as blood vessels, and other tissue architecture are potential future directions for this project. One of our goal was to be able to transform cancer from an acute and lethal disease that ultimately kills us to a chronic diseases that do not kill us. Our results suggest adaptive therapy might be able to achieve that goal and this transform healthcare.

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APPENDIX A

ACKNLOWEDGEMENT OF AUTHORSHIP: IN SILICO INVESTIGATIONS OF MULTI-DRUG ADAPTIVE THERAPY PROTOCOLS

In Silico Investigations of Multi-Drug Adaptive Therapy Protocols

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Conceptualization, C.C.M. and D.S.T.; methodology, D.S.T.; software, D.S.T.; statistical analysis, D.S.T.; writing—original draft preparation, D.S.T.; writing—review and editing, all authors; supervision, C.C.M., L.H.C. and A.R.A.A.; funding acquisition, C.C.M. and A.R.A.A. All authors have read and agreed to the published version of the manuscript.

APPENDIX B

SUPPLEMENTAL MATERIAL TO CHAPTER 3: IN SILICO INVESTIGATIONS OF MULTI-DRUG ADAPTIVE THERAPY PROTOCOLS

In this document I present:

1. Supplementary figures
2. Supplementary tables

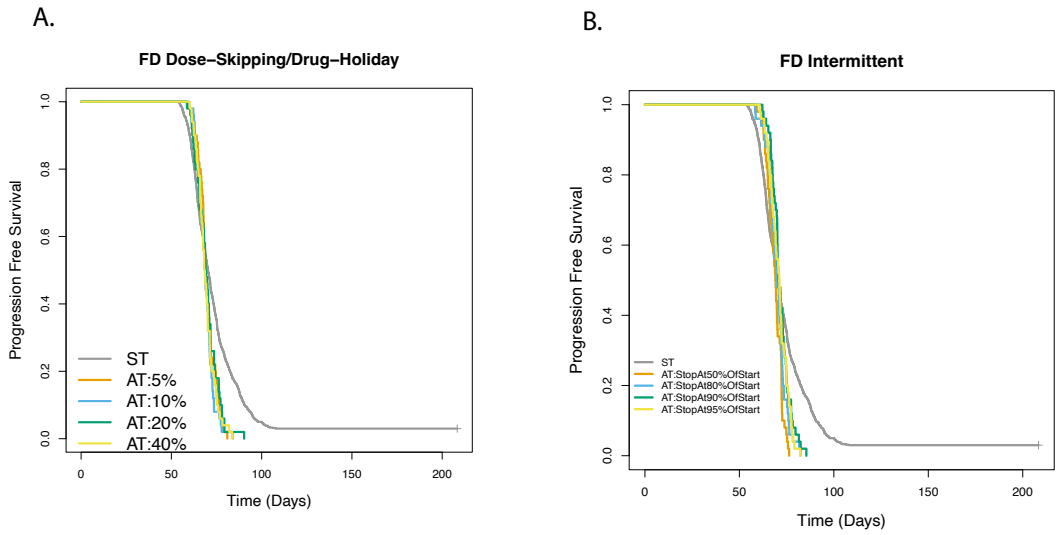

Figure S1. Role of Delta Tumor parameter in determining outcome of fixed-dose (FD) adaptive therapy protocols. For FD Dose-Skipping/Drug-Holiday, delta tumor is percentage change in tumor burden relative to the last measurement, such that a fixed dosage of the drugs is administered if the tumor burden exceeds the threshold, and treatment is skipped otherwise, default value being 10%. For FD Intermittent, Delta Tumor is the absolute value at which treatment is stopped relative to the baseline for treatment initiation, default being stopping treatment when tumor shrinks to 50% of the initial baseline for treatment initiation. The Fixed Dose (FD) protocols. **A.** FD Dose-Skipping/Drug-Holiday, **B.** FD intermittent are defined in the text.

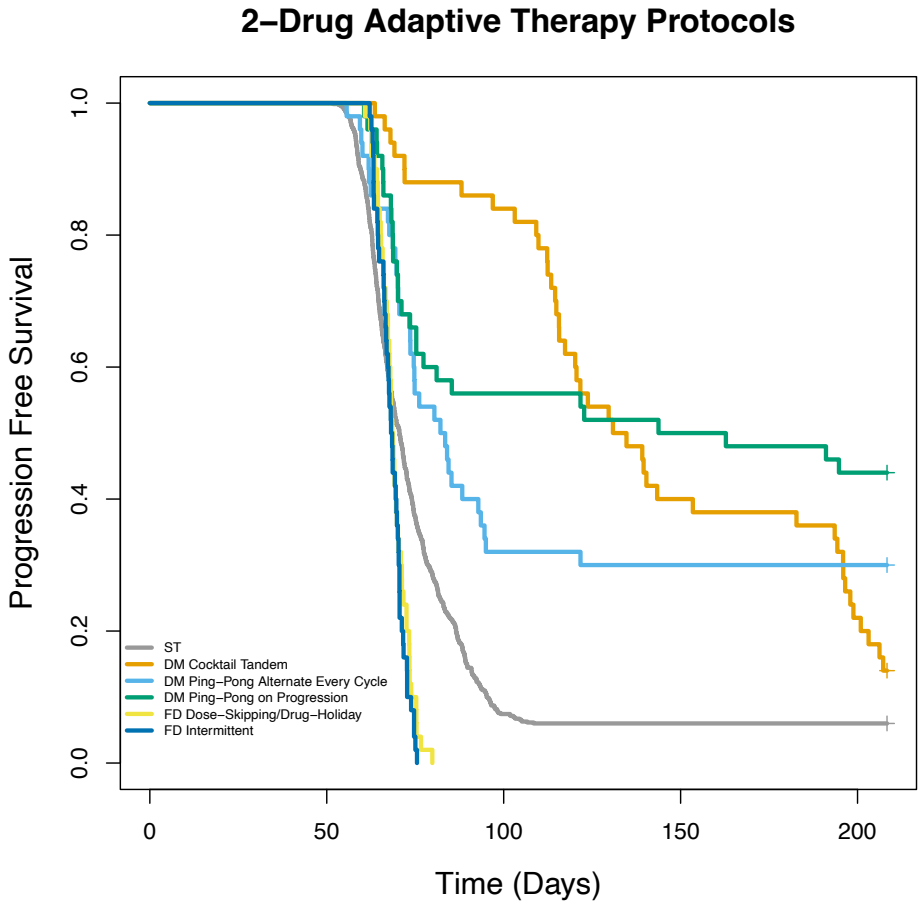

Figure S2. Survival curves when cells can undergo forward mutation to resistance phenotypes but no reverse mutations. Dose modulation (DM) adaptive therapy protocols still work better than fixed dose (FD) adaptive therapy protocols.

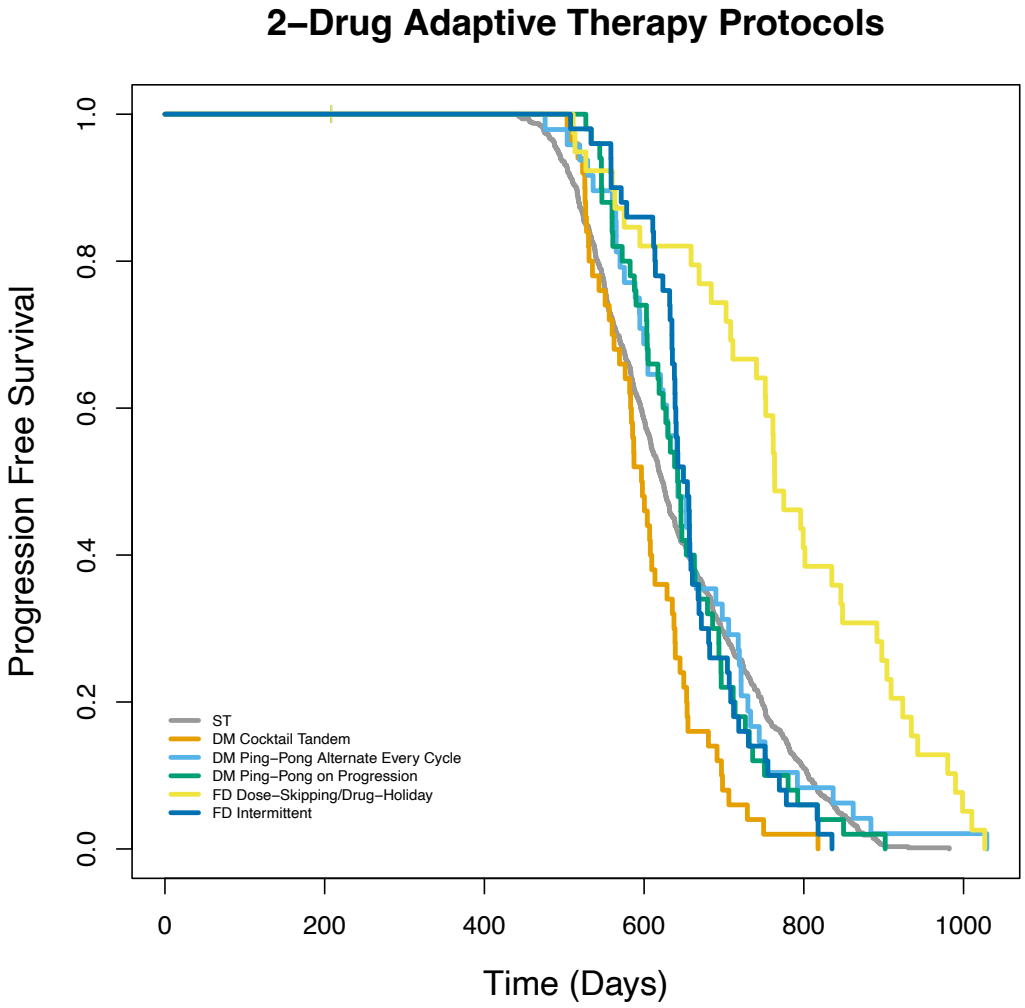

Figure S3. Survival curves when the doubling time of each cell type is increased by one order of magnitude relative to the default values, representing slow tumor doubling times. Note all therapies prevent progression for much longer than the 200 days we tested in previous experiments. Dose modulation (DM) adaptive therapy protocols and fixed dose (FD) adaptive therapy protocols are mostly equivalent to standard treatment (ST), though FD Dose-Skipping/Drug-Holiday is better, and DM Cocktail Tandem is worse.

Table S1: Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for various dose-modulation and fixed-dose adaptive therapy protocols relative to standard treatment (ST).

Protocol	Hazard Ratio (95% c.i.) relative to standard treatment	*p* value	Test for proportionality of hazards *p* value
DM Cocktail	0.25 (0.18-0.35)	<0.001	0.0016
DM Ping-Pong Alternate Every Cycle	0.26 (0.18-0.38)	<0.001	0.66
DM Ping-Pong on Progression	0.13 (0.08-0.22)	<0.001	0.045
FD Intermittent	1.67 (1.25-2.24)	<0.001	1.6e-10
FD Dose-Skipping/Drug Holiday	1.65 (1.23-2.21)	<0.001	1.2e-7

Table S2. Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for Fitness Cost Parameter. Abbreviations: n.s. is not significant, ST is standard treatment.

Comparison	Hazard Ratio (95% c.i.)	*p* value	Test for proportionality of hazards *p* value
ST (5X cost) relative to ST (3X cost)	1.47 (1.32-1.64)	<0.001	<2e-16
DM Cocktail (3X cost) relative to ST (3X cost)		n.s. (0.297)	

DM Cocktail (5X cost) relative to ST (5X cost)	0.25 (0.18-0.35)	<0.001	0.0016
DM Cocktail (5X cost) relative to DM Cocktail (3X cost)	0.09 (0.04-0.18)	<0.001	3.5e-8
DM Ping-Pong Alternate Every Cycle (3X cost) relative to ST (3X cost)		n.s. (0.706)	
DM Ping-Pong Alternate Every Cycle (5X cost) relative to ST (5X cost)	0.26 (0.18-0.38)	<0.001	0.66
DM Ping-Pong Alternate Every Cycle (5X cost) relative to DM Ping-Pong Alternate Every Dose (3X cost)	0.35 (0.21-0.56)	<0.001	3e-4
DM Ping-Pong on Progression (3X cost) relative to ST (3X cost)	1.34 (1.00-1.79)	0.0482	0.0052
DM Ping-Pong on Progression (5X cost) relative to ST (5X cost)	0.13 (0.08-0.22)	<0.001	0.045

DM Ping-Pong on Progression (5X cost) relative to DM Ping-Pong on Progression (3X cost)	0.17 (0.09-0.31)	<0.001	1.4e-8
FD Dose-Skipping/Drug Holiday (3X cost) relative to ST (3X cost)	1.47 (1.10-1.97)	0.00851	0.092
FD Dose-Skipping/Drug Holiday (5X cost) relative to ST (5X cost)	1.65 (1.23-2.21)	<0.001	1.2e-7
FD Dose-Skipping/Drug Holiday (5X cost) relative to FD Dose-Skipping (AT-2) (3X cost)	3.12 (2.03-4.80)	<0.001	0.23
FD Intermittent (3X cost) relative to ST (3X cost)		n.s. (0.344)	
FD Intermittent (5X cost) relative to ST (5X cost)	1.67 (1.25-2.24)	<0.001	1.6e-10
FD Intermittent (5X cost)	28.88 (12.73-65.54)	<0.001	0.17

relative to FD Intermittent (3X cost)			

Table S3. Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for Turnover Parameter. Abbreviations: n.s. is not significant, LT is low turnover, HT is high turnover, ST is standard treatment.

Comparison	Hazard Ratio (95% c.i.)	*p* value	Test for proportionality of hazards *p* value
ST (HT) relative to ST (LT)	0.68 (0.61-0.76)	<0.001	0.1
DM Cocktail (LT) relative to ST (LT)	0.25 (0.18-0.35)	<0.001	0.0018
DM Cocktail (HT) relative to ST (HT)	0.20 (0.13-0.30)	<0.001	3.7e-6
DM Cocktail (HT) relative to DM Cocktail (LT)	0.29 (0.17-0.49)	<0.001	0.62
DM Ping-Pong Alternate Every Cycle (LT) relative to ST (LT)	0.27 (0.19-0.40)	<0.001	0.31

DM Ping-Pong Alternate Every Cycle (HT) relative to ST (HT)	0.35 (0.24-0.51)	<0.001	0.032
DM Ping-Pong Alternate Every Cycle (HT) relative to DM Ping-Pong Alternate Every Dose (LT)		n.s. (0.424)	
DM Ping-Pong on Progression (LT) relative to ST (LT)	0.14 (0.09-0.22)	<0.001	0.93
DM Ping-Pong on Progression (HT) relative to ST (HT)	0.22 (0.14-0.34)	<0.001	0.072
DM Ping-Pong on Progression (HT) relative to DM Ping-Pong on Progression (LT)		n.s. (0.834)	

FD Dose-Skipping/Drug Holiday (LT) relative to ST (LT)	1.60 (1.19-2.14)	0.00175	2.1e-10
FD Dose-Skipping/Drug Holiday (HT) relative to ST (HT)	1.90 (1.42-2.55)	<0.001	8.7e-7
FD Dose-Skipping/Drug Holiday (HT) relative to FD Dose-Skipping (AT-2) (LT)	0.61 (0.39-0.93)	0.0234	0.0019
FD Intermittent (LT) relative to ST (LT)	1.58 (1.18-2.11)	0.00213	4.5e-7
FD Intermittent (HT) relative to ST (HT)	1.44 (1.07-1.92)	0.0151	1.5e-10
FD Intermittent (HT) relative to FD Intermittent (LT)	0.39 (0.26-0.60)	<0.001	0.25

Table S4. Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for Replacement Parameter. Abbreviations: n.s. is not significant, Rep is replacement, ST is standard treatment.

Comparison	Hazard Ratio (95% c.i.)	*p* value	Test for proportionality of hazards *p* value
ST (50% Rep) relative to ST (0% Rep)	0.36 (0.32-0.40)	<0.001	0.0016
ST (100% Rep) relative to ST (50% Rep)	0.83 (0.74-0.92)	<0.001	0.11
DM Cocktail (0% Rep) relative to ST (0% Rep)	0.32 (0.23-0.43)	<0.001	0.011
DM Cocktail (50% Rep) relative to ST (50% Rep)	0.25 (0.18-0.35)	<0.001	0.0016
DM Cocktail (100% Rep) relative to ST (100% Rep)	0.03 (0.01-0.08)	<0.001	0.097
DM Cocktail (50% Rep) relative to DM Cocktail (0% Rep)	0.08 (0.04-0.16)	<0.001	0.00033
DM Cocktail (100% Rep) relative to DM	0.05 (0.02-0.15)	<0.001	0.87

Cocktail (50% Rep)			
DM Ping-Pong Alternate Every Cycle (0% Rep) relative to ST (0% Rep)	0.28 (0.20-0.40)	<0.001	0.79
DM Ping-Pong Alternate Every Cycle (50% Rep) relative to ST (50% Rep)	0.26 (0.18-0.38)	<0.001	0.66
DM Ping-Pong Alternate Every Cycle (100% Rep) relative to ST (100% Rep)	0.23 (0.16-0.35)	<0.001	0.025
DM Ping-Pong Alternate Every Cycle (50% Rep) relative to DM Ping-Pong Alternate (0% Rep)	0.45 (0.27-0.73)	0.00128	0.028
DM Ping-Pong Alternate Every Cycle		n.s. (0.275)	

(100% Rep) relative to DM Ping-Pong Alternate (50% Rep)			
DM Ping-Pong on Progression (0% Rep) relative to ST (0% Rep)	0.24 (0.17-0.34)	<0.001	0.026
DM Ping-Pong on Progression (50% Rep) relative to ST (50% Rep)	0.13 (0.08-0.22)	<0.001	0.026
DM Ping-Pong on Progression (100% Rep) relative to ST (100% Rep)	0.07 (0.03-0.13)	<0.001	0.69
DM Ping-Pong on Progression (50% Rep) relative to DM Ping-Pong Progression (0% Rep)	0.23 (0.13-0.41)	<0.001	0.85
DM Ping-Pong on Progression (100% Rep) relative to DM	0.40 (0.17-0.92)	0.0305	0.044

Ping-Pong Progression (50% Rep)			
FD Dose-Skipping/Drug Holiday) (0% Rep) relative to ST (0% Rep)		n.s. (0.191)	
FD Dose-Skipping/Drug Holiday (50% Rep) relative to ST (50% Rep)	1.65 (1.23-2.21)	<0.001	1.2e-7
FD Dose-Skipping/Drug Holiday (100% Rep) relative to ST (100% Rep)		n.s. (0.938)	
FD Dose-Skipping/Drug Holiday (50% Rep) relative to FD Dose-Skipping (AT-2) (0% Rep)	0.31 (0.21-0.48)	<0.001	0.002
FD Dose-Skipping/Drug Holiday (100% Rep) relative to FD Dose-	0.26 (0.17-0.41)	<0.001	0.47

Skipping (AT-2) (50% Rep)			
FD Intermittent (0% Rep) relative to ST (0% Rep)	1.49 (1.12-1.99)	<0.00696	0.00043
FD Intermittent (50% Rep) relative to ST (50% Rep)	1.67 (1.25-2.24)	<0.001	1.6e-10
FD Intermittent (100% Rep) relative to ST (100% Rep)		n.s. (0.294)	
FD Intermittent (50% Rep) relative to FD Intermittent (0% Rep)	0.15 (0.09-0.25)	<0.001	0.089
FD Intermittent (100% Rep) relative to FD Intermittent (50% Rep)	0.25 (0.16-0.40)	<0.001	0.96

Table S5. Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for Delta Tumor Parameter. Abbreviations: n.s. is not significant, ST is standard treatment.

Comparison	Hazard Ratio (95% c.i.)	*p* value	Test for proportionality of hazards *p* value
DM Cocktail (Delta Tumor 5%) relative to ST		n.s. (0.982)	
DM Cocktail (Delta Tumor 10%) relative to ST	0.25 (0.18-0.35)	<0.001	0.0016
DM Cocktail (Delta Tumor 20%) relative to ST	0.57 (0.43-0.76)	<0.001	3.2e-7
DM Cocktail (Delta Tumor 40 %) relative to ST		n.s. (0.166)	
DM Cocktail (Delta Tumor 10%) relative to DM Cocktail (Delta Tumor 5%)		n.s. (0.996)	
DM Cocktail (Delta Tumor 20%) relative to DM Cocktail (Delta Tumor 10%)	11.68 (5.61-24.32)	<0.001	7e-9

DM Cocktail (Delta Tumor 40%) relative to DM Cocktail (Delta Tumor 20%)	6.73 (3.74-12.11)	<0.001	4.3e-5
DM Ping-Pong Alternate Every Cycle (Delta Tumor 5%) relative to ST	0.31 (0.22-0.44)	<0.001	0.5
DM Ping-Pong Alternate Every Cycle (Delta Tumor 10%) relative to ST	0.26 (0.18-0.38)	<0.001	0.66
DM Ping-Pong Alternate Every Cycle (Delta Tumor 20%) relative to ST	0.50 (0.37-0.68)	<0.001	0.053
DM Ping-Pong Alternate Every Cycle (Delta Tumor 40 %) relative to ST	0.67 (0.50-0.90)	0.00795	4.2e-7
DM Ping-Pong Alternate Every Cycle (Delta Tumor 10%) relative to DM Ping-Pong Alternate Every Cycle (Delta Tumor 5%)		n.s. (0.397)	

DM Ping-Pong Alternate Every Cycle (Delta Tumor 20%) relative to DM Ping-Pong Alternate Every Cycle (Delta Tumor 10%)	1.97 (1.22-3.18)	0.00571	0.097
DM Ping-Pong Alternate Every Cycle (Delta Tumor 40%) relative to DM Ping-Pong Alternate Every Cycle (Delta Tumor 20%)		n.s. (0.0517)	
DM Ping-Pong on Progression (Delta Tumor 5%) relative to ST	0.09 (0.05-0.16)	<0.001	0.0018
DM Ping-Pong on Progression (Delta Tumor 10%) relative to ST	0.13 (0.08-0.22)	<0.001	0.045
DM Ping-Pong on Progression (Delta Tumor 20%) relative to ST	0.40 (0.29-0.55)	<0.001	0.82
DM Ping-Pong on Progression		n.s. (0.545)	

(Delta Tumor 40 %) relative to ST			
DM Ping-Pong on Progression (Delta Tumor 10%) relative to DM Ping-Pong on Progression (Delta Tumor 5%)		n.s. (0.356)	
DM Ping-Pong on Progression (Delta Tumor 20%) relative to DM Ping-Pong on Progression (Delta Tumor 10%)	3.20 (1.80-5.68)	<0.001	0.018
DM Ping-Pong on Progression (Delta Tumor 40%) relative to DM Ping-Pong on Progression (Delta Tumor 20%)	2.71 (1.71-4.28)	<0.001	0.022
FD Dose-Skipping/Drug Holiday (Delta Tumor 5%) relative to ST	1.50 (1.12-2.01)	0.00631	4.2e-8
FD Dose-Skipping/Drug Holiday (Delta	1.65 (1.23-2.21)	<0.001	1.2e-7

Tumor 10%) relative to ST			
FD Dose-Skipping/Drug Holiday (Delta Tumor 20%) relative to ST	1.45 (1.11-1.98)	0.00832	0.00015
FD Dose-Skipping/Drug Holiday (Delta Tumor 40 %) relative to ST	1.59 (1.18-2.12)	0.00196	1.6e-6
FD Dose-Skipping/Drug Holiday (Delta Tumor 10%) relative to FD Dose-Skipping (AT-2) (Delta Tumor 5%)		n.s. (0.484)	
FD Dose-Skipping/Drug Holiday (Delta Tumor 20%) relative to FD Dose-Skipping (AT-2) (Delta Tumor 10%)		n.s. (0.254)	
FD Dose-Skipping/Drug Holiday (Delta Tumor 40%) relative to FD Dose-Skipping (AT-2) (Delta Tumor 20%)		n.s. (0.51)	

FD Intermittent (Stop At 50% of Start) relative to ST	1.67 (1.25-2.24)	<0.001	1.6e-10
FD Intermittent (Stop At 80% of Start) relative to ST	1.45 (1.08-1.94)	0.0127	1.6e-8
FD Intermittent (Stop At 90% of Start) relative to ST		n.s. (0.154)	
FD Intermittent (Stop At 95% of Start) relative to ST		n.s. (0.0558)	
FD Intermittent (Stop At 80% of Start) relative to FD Intermittent (Stop At 50% of Start)		n.s. (0.0767)	
FD Intermittent (Stop At 90% of Start) relative to FD Intermittent (Stop At 80% of Start)		n.s. (0.13)	
FD Intermittent (Stop At 95% of Start) relative to FD		n.s. (0.432)	

Intermittent (Stop At 90% of Start)			

Table S6. Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for Delta Dose Parameter. Abbreviations: n.s. is not significant, ST is standard treatment.

Comparison	Hazard Ratio (95% c.i.)	*p* value	Test for proportionality of hazards *p* value
DM Cocktail (Delta Dose 25%) relative to ST		n.s. (0.355)	
DM Cocktail (Delta Dose 50%) relative to ST	0.34 (0.24-0.47)	<0.001	1.2e-5
DM Cocktail (Delta Dose 75%) relative to ST	0.40 (0.29-0.54)	<0.001	3.3e-9
DM Cocktail (Delta Dose 50%) relative to DM Cocktail (Delta Dose 25%)	0.09 (0.04-0.18)	<0.001	1.2e-6
DM Cocktail (Delta Dose 75%) relative to DM Cocktail	1.74 (1.13-2.67)	0.0112	0.68

(Delta Tumor 50%)			
DM Ping-Pong Alternate Every Cycle (Delta Dose 25%) relative to ST	0.37 (0.26-0.53)	<0.001	0.79
DM Ping-Pong Alternate Every Cycle (Delta Dose 50%) relative to ST	0.46 (0.33-0.64)	<0.001	0.0018
DM Ping-Pong Alternate Every Cycle (Delta Dose 75%) relative to ST	0.39 (0.27-0.55)	<0.001	0.069
DM Ping-Pong Alternate Every Cycle (Delta Dose 50%) relative to DM Ping-Pong Alternate Every Cycle (Delta Dose 25%)		n.s. (0.306)	
DM Ping-Pong Alternate Every Cycle (Delta Dose 75%) relative to DM Ping-Pong Alternate Every Cycle (Delta Tumor 50%)		n.s. (0.451)	

DM Ping-Pong on Progression (Delta Dose 25%) relative to ST	0.13 (0.08-0.23)	<0.001	0.021
DM Ping-Pong on Progression (Delta Dose 50%) relative to ST	0.15 (0.09-0.25)	<0.001	0.00064
DM Ping-Pong on Progression (Delta Dose 75%) relative to ST	0.29 (0.20-0.44)	<0.001	0.085
DM Ping-Pong on Progression (Delta Dose 50%) relative to DM Ping-Pong on Progression (Delta Dose 25%)		n.s. (0.754)	
DM Ping-Pong on Progression (Delta Dose 75%) relative to DM Ping-Pong on Progression (Delta Tumor 50%)		n.s. (0.0795)	

Table S7. Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for Treatment Vacation Parameter. Abbreviations: n.s. is not significant, ST is standard treatment.

Comparison	Hazard Ratio (95% c.i.)	*p* value	Test for proportionality of hazards *p* value
DM Cocktail (Treat Vacation at 10% of start) relative to ST	1.95 (1.45-2.61)	<0.001	0.00014
DM Cocktail (Treat Vacation at 50% of start) relative to ST	0.25 (0.18-0.35)	<0.001	0.0016
DM Cocktail (Treat Vacation at 80% of start) relative to ST	0.19 (0.13-0.26)	<0.001	2.1e-8
DM Cocktail (Treat Vacation at 50% of start) relative to DM Cocktail (Treat Vacation at 10% of start)	0.05 (0.02-0.11)	<0.001	0.019
DM Cocktail (Treat Vacation at 80% of start) relative to DM Cocktail (Treat Vacation at 50% of start)		n.s. (0.0966)	
DM Ping-Pong Alternate Every Cycle (Treat Vacation at 10% of start) relative to ST		n.s.(0.277)	

DM Ping-Pong Alternate Every Cycle (Treat Vacation at 50% of start) relative to ST	0.26 (0.18-0.38)	<0.001	0.66
DM Ping-Pong Alternate Every Cycle (Treat Vacation at 80% of start) relative to ST	0.19 (0.13-0.28)	<0.001	0.0031
DM Ping-Pong Alternate Every Cycle (Treat Vacation at 50% of start) relative to DM Ping-Pong Alternate Every Cycle (Treat Vacation at 10% of start)	0.25 (0.15-0.40)	<0.001	0.46
DM Ping-Pong Alternate Every Cycle (Treat Vacation at 80% of start) relative to DM Ping-Pong Alternate Every Cycle (Treat Vacation at 50% of start)		n.s. (0.31)	
DM Ping-Pong on Progression (Treat Vacation at 10% of start) relative to ST	1.38 (1.03-1.84)	0.0311	0.096

DM Ping-Pong on Progression (Treat Vacation at 50% of start) relative to ST	0.13 (0.08-0.22)	<0.001	0.045
DM Ping-Pong on Progression (Treat Vacation at 80% of start) relative to ST	0.16 (0.11-0.25)	<0.001	0.19
DM Ping-Pong on Progression (Treat Vacation at 50% of start) relative to DM Ping-Pong on Progression (Treat Vacation at 10% of start)	0.13 (0.07-0.24)	<0.001	0.73
DM Ping-Pong on Progression (Treat Vacation at 80% of start) relative to DM Ping-Pong on Progression (Treat Vacation at 50% of start)		n.s. (0.76)	

Table S8: Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for various dose-modulation and fixed-dose adaptive therapy protocols relative to standard treatment (ST) under conditions when cells can undergo forward mutations only but no reverse mutations.

Protocol	Hazard Ratio (95% c.i.) relative to standard treatment	*p* value	Test for proportionality of hazards *p* value
DM Cocktail	0.33 (0.24-0.45)	<0.001	1.3e-7
DM Ping-Pong Alternate Every Cycle	0.46 (0.33-0.64)	<0.001	0.59
DM Ping-Pong on Progression	0.30 (0.20-0.44)	<0.001	0.38
FD Intermittent	1.79 (1.33-2.40)	<0.001	1.1e-12
FD Dose-Skipping/Drug Holiday	1.67 (1.24-2.24)	<0.001	9.1e-12

Table S9: Hazard ratios along with the 95% confidence intervals (c.i.), *p*-values, and test for proportionality of hazards *p*-values for various dose-modulation and fixed-dose adaptive therapy protocols relative to standard treatment (ST) under conditions when the doubling time of each cell type is increased by one order of magnitude relative to the default values.

Protocol	Hazard Ratio (95% c.i.) relative to standard treatment	*p* value	Test for proportionality of hazards *p* value
DM Cocktail	1.62 (1.21-2.17)	0.0011	0.016
DM Ping-Pong Alternate Every Cycle	0.88 (0.66-1.19)	n.s. (0.42)	0.33

DM Ping-Pong on Progression	1.00 (0.75-1.33)	n.s. (0.997)	0.018
FD Intermittent	1.00 (0.75-1.34)	n.s. (0.997)	1.7e-6
FD Dose-Skipping/Drug Holiday	0.31 (0.21-0.44)	<0.001	0.95

APPENDIX C

CONTRIBUTIONS TO CHAPTERS 2, 4, & 5

All of the work carried out in this dissertation is either published or soon to be published. I would like to outline the author contributions to the manuscripts presented within these works. I am grateful for collaborations with these people: Carlo Casebier Maley (C.C.M), Luis H. Cisneros (L.H.C), Alexander R. A. Anderson (A.R.A.A), Sareh Seyedi (S.S.). I have used multiple names during the course of this dissertation: D.S.T refers to Daniel Saha Thomas (D.S.T.) and D.K.S. refers to Daniel Kaushik Saha.

For Chapter 2: Adaptive Therapy Using a Single Cytotoxic or a Single Cytostatic Drug Conceptualization, C.C.M. and D.K.S.; methodology, D.K.S.; software, D.K.S.; statistical analysis, D.K.S.; writing—original draft preparation, D.K.S.; writing—review and editing, all authors; supervision, C.C.M., L.H.C. and A.R.A.A.; funding acquisition, C.C.M. and A.R.A.A. All co-authors have granted their permission for the use of the works in this dissertation.

For Chapter 4: Adaptive Therapy Using Two Cytotoxic or Two Cytostatic Drugs Conceptualization, C.C.M. and D.K.S.; methodology, D.K.S.; software, D.K.S.; statistical analysis, D.K.S.; writing—original draft preparation, D.K.S.; writing—review and editing, all authors; supervision, C.C.M., L.H.C. and A.R.A.A.; funding acquisition, C.C.M. and A.R.A.A. All co-authors have granted their permission for the use of the works in this dissertation.

For Chapter 5: Comparison of In Silico Modeling Results to Preclinical Mice Experiments

Conceptualization, C.C.M. and D.K.S.; methodology, D.K.S.; software, D.K.S.; statistical analysis, D.K.S.; writing—original draft preparation, D.K.S.; writing—review and editing, all authors; supervision, C.C.M., L.H.C. and A.R.A.A.; funding acquisition, C.C.M. and A.R.A.A. Experimental data produced by S.S. All co-authors have granted their permission for the use of the works in this dissertation.