Turnover modulates the need for a cost of resistance in adaptive therapy

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1 Abstract

"Control and conquer" - this is the philosophy behind adaptive therapy (AT), which seeks to exploit intra-tumoral competition to avoid, or at least, delay the emergence of therapy resistance in cancer. Motivated by promising results from theoretical, experimental and, most recently, clinical studies, there is an increasing interest in extending this approach to other cancers. As such, it is urgent to understand the characteristics of a cancer which determine whether it will respond well to AT, or not. A plausible candidate for such a selection criterion is the fitness cost of resistance. In this paper, we study a 2-population ordinary differential equation (ODE) model to investigate whether the presence of a cost is a necessary condition for AT to extend the time to progression beyond that of a standard-of-care continuous therapy. We find that for tumours close to their environmental carrying capacity such a cost of resistance is not required. However, for tumours growing far from carrying capacity a cost may be required to see meaningful gains. Notably, we show that in such cases it is important to consider the cell turnover in the tumour and we discuss its role in modulating the impact of a cost of resistance. Overall, our work helps to clarify under which circumstances AT may be beneficial, and suggests that turnover may play an unexpectedly important role in the decision making process.

2 Introduction

The evolution of drug resistance is one of the biggest challenges in cancer therapy. In 1947, Farber et al [15] for the first time reported that children suffering from acute leukemia, a hitherto untreatable disease, had shown "significant improvements" [15] upon treatment with a chemical agent, 4-aminopteroylglutamic acid. However, they also also noted that these remissions were temporary [15]. This pattern still holds true today for many patients, and applies to both chemo- as well as targeted therapies.

Research aiming to combat drug-resistance has traditionally focussed on developing drugs which target either the resistance mechanism or kill the cell through a different route. As an alternative to this molecular approach, a number of authors (most notably Gatenby and colleagues [19, 20, 12], but also [34] and [24]) have proposed that resistance could be delayed, if not averted, through changes in drug-scheduling. The design principle behind current treatment schedules is to maximise cell kill by treating at the maximum tolerated dose (MTD) in as continuous a fashion as toxicity permits. However, if drug-resistant cells are present *a priori* or develop during treatment, such aggressive treatment releases these cells from the competition for space and resources and facilitates their growth, a process known as "competitive release" [34, 20, 19]. Inspired by approaches used in the management of invasive species and agricultural pests, Gatenby et al [19, 20] proposed adaptive therapy (AT) which aims not to eradicate the tumour, but to control it. Therapy is applied to reduce tumour burden to a tolerable level but is subsequently modulated or withdrawn to maintain a pool of drug-sensitive cancer cells. Over the past ten years, a number of studies have shown that AT can extend time to progression (TTP) *in vivo*, first in ovarian [20], and breast cancer [14], and most recently in melanoma [39]. Moreover, the interim analysis of the first human trial of AT, which applied AT to the treatment of metastatic castrate-resistant prostate cancer with androgen-deprivation therapy, reported an increase in TTP of at least 27 months and a reduction in cumulative drug usage of 47% [47]. While it is challenging to prove that competition is the mechanism through which AT extends TTP, and not, for example, synergistic effects on vasculature or the immune system, some evidence exists. Bacevic et al [3] showed that low dose treatment was able to minimise the frequency of resistant cells for longer than high dose treatment in a spheriod model of cyclin-dependent kinase inhibitor treatment in colorectal cancer, which has neither vasculature nor an immune system. Moreover, they found that this was not true if they repeated the experiment in a 2-d cell-culture model in which cells compete less strongly [3]. Furthermore, Smalley et al [39] observed that in both mouse and human samples of melanoma there was an enrichment for cells with a drug-sensitive transcriptional signature if the tumours had previously experienced a drug holiday, as would be predicted by the competitive control hypothesis.

An important topic in the development of novel therapies is the identification of patient selection criteria. The success in prostate cancer has spurred interest in extending AT to other cancers such as thyroid cancer or melanoma (clinicaltrials.gov identifiers NCT03630120 and NCT03543969, respectively). As such, it is urgent to understand the characteristics of a cancer which determine whether it will respond well to AT, or not. Bacevic et al [3] showed through *in vitro* experiments and an agent-based computational model that it is important that cells are spatially constrained, as otherwise the competition is too weak to effectively control the growth of resistant cells. Furthermore, through ODE modelling they found that the fitness of the resistant population when the population is rare is a key determinant of the benefit derived from AT [3]. These results were corroborated by Gallaher et al [17] who compared two AT

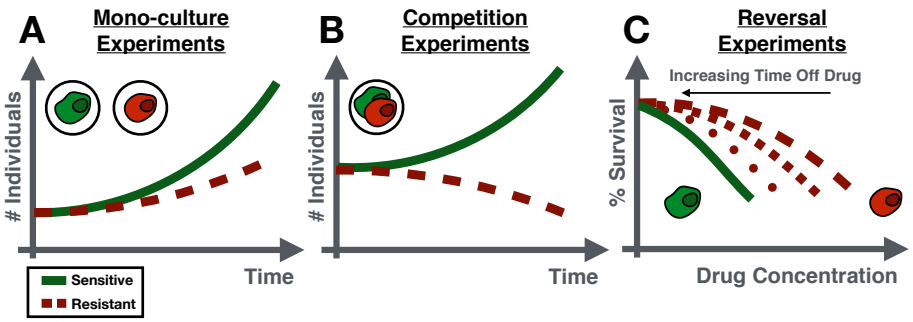


Figure 1: *The three main experimental designs typically used to test for a cost of resistance. All experiments are carried out in the absence of drug, either in in vitro or in vivo. A) Mono-culture experiments study the resistant strain in isolation, examining changes in growth rate, size, migration rate etc. B) Competition experiments compare the abundance of sensitive and resistant cells over time. The higher the cost, the faster the resistant population is expected to disappear. C) Reversal experiments examine the rate at which the drug resistance is lost, if the drug is withdrawn. To do so, the resistant population is cultured in a drug-free environment and its drug response is tested at regular time intervals.*

strategies on an agent-based model which modelled resistance as a continuous trait. They found that the initial abundance of resistance, the rate of spatial mixing through migration, and the rate of acquisition of resistance through mutation, were the key factors in determining the benefit from AT, and which AT strategy was most effective. Using a non-spatial game theory model, West et al [44] showed that from a mathematical perspective the benefit of AT is determined by the relative position of the initial tumour to the nullcline of the resistant population in phase space. Biologically, this means that the stronger the selection against resistance in the absence of drug, the longer AT can be expected to extend TTP. Finally, while not directly focussed on cancer, Hansen et al [24] present a general argument for how to decide when treatment should aim to control and when it should aim to eradicate a pathogen. They derive quantitative expressions for the levels of initial resistance above or below which a management strategy such as AT would perform better than an eradication strategy [24].

However, a factor which is still poorly understood is the role of the cost of resistance. *"Cost of resistance"* refers to the notion that a resistance adaptation not only confers a fitness advantage under drug exposure, but that it might also come at the *cost* of a fitness disadvantage in a treatment-free environment [19, 30]. It is a phenomenon which has been widely studied in the agricultural pests and antibiotic resistance communities [2, 1] and is typically experimentally measured in one of three ways: i) Mono-culture experiments, which measure the growth rate of the resistant population in the absence of drug in isolation, ii) Competition experiments, which study the relative fre-

quency of sensitive and resistant cells over time when cultured together, and iii) Reversal experiments, which explore how long it takes for resistant cells to lose their resistance mechanism if grown in absence of drug for long times (Figure 1). For example, Gallaher et al [17] report that the doubling time of doxorubicin resistant MCF7DOX breast cancer cells is increased by 50% compared to their sensitive counterparts, and that in competition experiments the sensitive cells outcompete the resistant cells in absence of the drug. This is likely because these cells use P Glycoprotein-related efflux pumps to resist treatment, for which they have to divert energy away from proliferation towards running of the pumps [9, 17]. Bacevic et al [3] make similar observations for CDKi resistant cells and a number of further examples can be found in the literature (e.g. [27, 5, 26, 10, 28]). In their original work on AT Gatenby et al [20, 19] used the cost of resistance to motivate AT, and most theoretical studies since have made this assumption [3, 17, 44]. However, not all resistance mechanisms come at a cost. In fact, Behrens et al [4] find that A2780 cis-platin resistant ovarian cancer cells have a shorter doubling time than the sensitive parental line (22.1h compared to 25.3h). Similar observations have been made for certain collorectal cancer cell lines [26], and Kaznatcheev et al [28] report that even though their resistant cells grow more slowly in mono-culture, their growth is supported by the presence of sensitive cells in co-culture [28]. In addition, despite the presence of a cost of resistance Bacevic et al [3] find that resistance is maintained for over 6 months in their reversal experiments, an observation also made elsewhere [27, 5, 26]. Given this wide range of experimentally observed cost values, it is necessary to identify the precise relationship between cost of resistance and the success of AT.

The aim of this paper is to clarify the impact of a cost of resistance on AT. We use a simple mathematical model in which we divide the tumour into drug-sensitive and drug-resistant cells and model their growth with two ordinary differential equations (ODEs). We compare the TTP under standard-of-care continuous therapy (CT) with that of the AT algorithm used in the clinical trial by Zhang et al [47], first in the absence, and subsequently in the presence, of a cost of resistance. We find that the ability of AT to control resistance depends on how close the resistant population is to its population equilibrium at the start of treatment. Importantly, this equilibrium is determined both by environmental carrying capacity as well as by the population's turnover. We conclude that turnover is a key factor to consider, not only to understand the impact of a cost of resistance, but also to assess the ability to control a tumour with AT in more general.

3 The Mathematical Model

In order to understand how we can use AT to control drug resistance, we will focus on tumour growth dynamics in a region of tissue which is sufficiently small so that we can assume cells experience approximately the same environmental conditions and we can neglect differences in the spatial distribution of cells. We

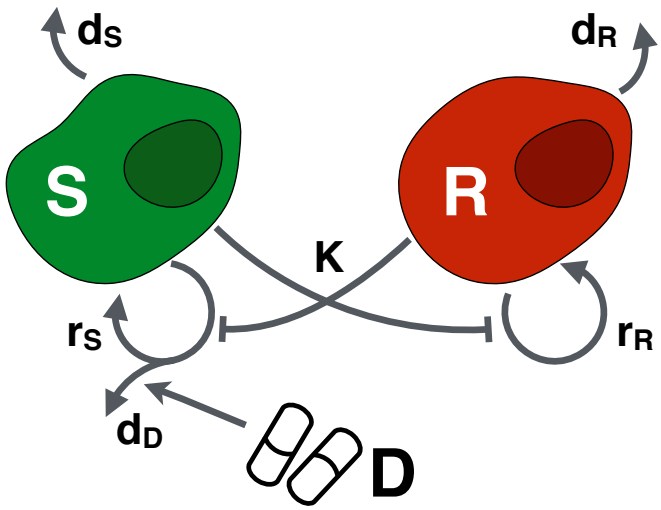


Figure 2: *The mathematical model. Drug-sensitive (S) and resistant cells (R) proliferate and die at rates, r_S and r_R, and d_S and d_R, respectively. Proliferating sensitive cells die at a rate d_D when exposed to drug, D. Finally, both populations compete for resources, where K denotes the total (shared) environmental carrying capacity.*

will assume that the initial density of tumour cells is given by N_0 and that the subsequent tumour cell density in the tissue over time, t, can be described by the continuous function, $N(t)$. Similarly, we will denote the concentration of drug over time as $D(t)$.

Tumours are heterogeneous populations of cells that differ in both their genetics and epigenetics [32]. As a result, even before a drug is given there is potential for some tumour cells to respond at a lower rate than the majority of the population, showing a degree of pre-existing resistance [25, 22, 21]. These cells will persist longer than their more sensitive counterparts and provide the founding pool that, over multiple rounds of selection and potential acquisition of further resistance mechanisms, can give rise to a fully drug-resistant cancer population [25, 38].

To model tumour heterogeneity, we will assume that the tumour is composed of tumour cell types with different levels of drug sensitivity. In the spirit of keeping our model simple we will assume that we can divide the tumour into drug-sensitive cells, $S(t)$, and cells which are fully resistant, $R(t)$. Furthermore, we will make the following assumptions about tumour growth:

- The initial densities of sensitive and resistant cells are given by S_0 and R_0, respectively.
- In isolation each population grows logistically with intrinsic growth rates r_S and r_R and with shared carrying capacity K_S and K_R, respectively.

Furthermore, cells die at natural death rates $d_S < r_S$ and $d_R < r_R$, respectively.

- The tumour cells compete with each other for resources and space according to a Lotka-Volterra functional response. This means that the presence of the competitor reduces a population's growth rate in a fashion that is linearly proportional to the competitor's population density. For simplicity we will assume that the strength of inter- and intra-species competition are the same.

- Only actively dividing cells are killed by the drug. Many chemotherapies induce DNA damage or inhibit the division machinery, which induces apoptosis only in cells that attempt to divide [43]. We will adopt the classical Norton-Simon model [35] which assumes that cell kill increases linearly with the drug dose, $D(t)$, so that at MTD a fraction d_D of dividing cells are killed.

- To facilitate our analysis we will make the simplifying assumption that the cost of resistance manifests itself solely in the growth rate r_R. This is also the most common way in which the cost is modelled (e.g. [3, 17, 22]). For a discussion of the implications of other manifestations of a cost, see Section A1.2 of the Appendix. As such we will assume that $d_S = d_R := d_T$, and $K_S = K_R := K$.

This translates into the following set of ODEs:

$$\frac{dS}{dt} = r_S \left(1 - \frac{S+R}{K}\right)\left(1 - \frac{2d_D}{D_{Max}} D(t)\right) S - d_T S, \tag{1}$$

$$\frac{dR}{dt} = r_R \left(1 - \frac{R+S}{K}\right) R - d_T R, \tag{2}$$

$$N(t) = S(t) + R(t), \tag{3}$$

with initial conditions:

$$N(0) = N_0, \quad S(0) = S_0, \quad \text{and} \quad R(0) = R_0,$$

where $N_0 = S_0 + R_0$. Note that the factor of 2 in the drug response comes from the fact that if a cell dies during mitosis not only the potential daughter but also the mother are lost. We observe also that the total tumour density is fully determined by the density of sensitive and resistant cells, so that it suffices to study Equations (1) and (2) to obtain $N(t)$. Thus, in the following we will primarily focus on Equations (1) and (2). For a mathematical discussion of the steady states of this system, see Section A1.1 of the Appendix.

The current drug concentration, $D(t)$, is determined by the treatment schedule and the drug's pharmacokinetics. For simplicity, we will assume that the latter can be neglected and the concentration over time follows exactly the levels foreseen by the drug schedules. We will consider two treatment schedules:

1. CT at the MTD, D_{Max}: $D(t) = D_{Max} \forall\, t$.

2. AT which withdraws treatment once a 50% decrease from the initial tumour size is achieved:

$$D(t) = \begin{cases} D_{\text{Max}}, & \text{until } N(t) < 50\% N_0 \\ 0, & \text{until } N(t) = N_0 \end{cases} \tag{4}$$

3.1 Non-dimensionalisation

In order to reduce the number of free variables we will non-dimensionalise Equations (1) - (3). Given we are interested in studying the treatment dynamics if we change the characteristics of the resistant cells relative to the sensitive cells, we base our scales on the sensitive population. Specifically, we will use the following transformations:

$$\tau = r_S t, \quad s = \frac{S}{K}, \quad r = \frac{R}{K}, \quad c = \frac{D}{D_{\text{Max}}}, \quad \text{and} \quad n = \frac{N}{K}.$$

This yields:

$$\frac{ds}{d\tau} = (1 - s - r)\,(1 - \hat{d}_D\, c(\tau))\, s - \hat{d}_T s, \tag{5}$$

$$\frac{dr}{d\tau} = \hat{r}_R\,(1 - s - r)\, r - \hat{d}_T r, \tag{6}$$

$$n(\tau) = s(\tau) + r(\tau), \tag{7}$$

where:

$$\hat{d}_D = 2d_D, \quad \hat{d}_T = \frac{d_T}{r_S}, \quad \text{and} \quad \hat{r}_R = \frac{r_R}{r_S}.$$

The initial conditions transform to:

$$n(0) = \frac{N_0}{K} := n_0, \quad s(0) = \frac{S_0}{K} := s_0, \quad \text{and} \quad r(0) = \frac{R_0}{K} := r_0,$$

and the treatment schedules are adjusted accordingly as well (not shown). Finally, for notational convenience we will define $f_S := s_0/n_0$ and $f_R := r_0/n_0$ as the initial fractions of sensitive and resistant cells, respectively.

3.2 Parameters

Given the key role which prostate cancer has played in the development of AT we parametrise our model according to this disease. As such, we adopt the proliferation rate for sensitive cells given in [47] ($r_S = 0.027\text{d}^{-1}$) as our time scale and the drug kill parameter $\hat{d}_D = 1.5$ from [46]. For the other parameters we perform parameter sweeps within their biologically realistic ranges. All parameters, their definitions and their ranges are summarised in Table 1.

Table 1: Parameters used in the numerical simulation of the model.

Parameter	Description	Value	Reference
r_S	Sensitive cell proliferation rate and model time scale	$0.027d^{-1}$	Adopted from [47].
$\hat{r}_R := \frac{r_R}{r_S}$	Resistant cell proliferation rate (normalised)	0.5 - 1.0	Lower limit: [17]; Upper limit: assumption of no cost
$\hat{d}_T := \frac{d_T}{r_S}$	Cell turnover rate (normalised)	0.0 - 0.3	Lower limit: assumption of no turnover; Upper limit: conservative estimate based on [40, 31].
$\hat{d}_D := 2d_D$	Drug-induced cell kill factor. Corresponds to twice the probability that a dividing cell will be killed at MTD.	1.5	Adopted from [46].
$n_0 := \frac{N_0}{K}$	Initial tumour cell density (normalised)	0.1 - 0.75	Parameter sweep; values within this range reported by [37].
$f_R := \frac{R_0}{N_0}$	Initial resistant cell fraction	0.001 - 0.1	Parameter sweep; values within this range reported by [21].
$f_S := \frac{S_0}{N_0}$	Initial sensitive cell fraction	0.9 - 0.999	Determined by $1 - f_R$.

3.3 Numerical Methods

The equations were solved using the RK5(4) explicit Runge-Kutta scheme [13] provided in Scipy (specifically, the SCIPY.INTEGRATE.ODE class). AT was implemented by simulating an interval $[t, t+\Delta t]$ at a time, and subsequently updating the dose for the next interval according to the algorithm in Equation 4. In preliminary experiments we tested different values of Δt and found $\Delta t = r_S$ to provide a good trade-off between speed and accuracy (not shown). All simulations were carried out in Python 3.6, using Scipy 1.1.0 and Numpy 1.15.1. Visualisations were produced with Pandas 0.23.4, Matplotlib 2.2.3, and Seaborn 0.9.0. The code will be made available on-line upon publication.

4 Results

In advanced cancers curative approaches are rarely successful. Instead, treatment success is often defined by how long therapy can prevent the tumour from progressing beyond a certain size. In this paper we seek to clarify the impor-

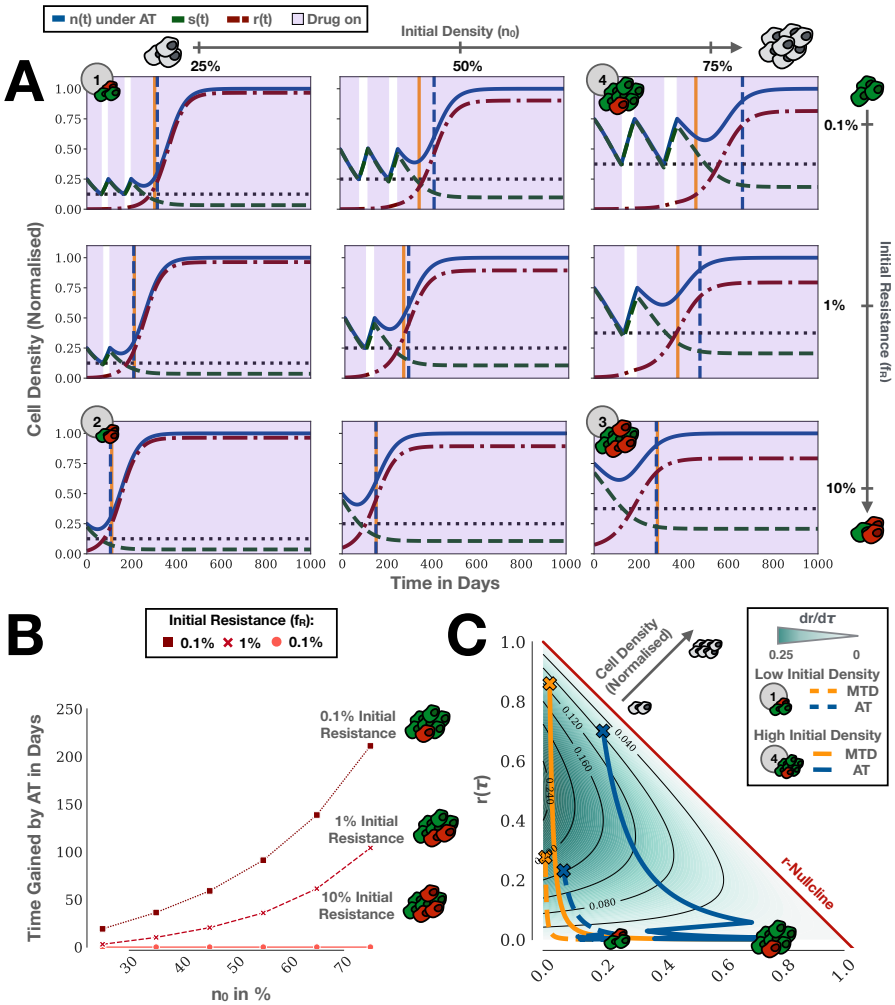


Figure 3: *AT can extend TTP compared to CT even in the absence of a cost of resistance. For simplicity, we are assuming no turnover here ($\hat{d}_T = 0$). A) Simulations of AT for a cohort of tumours with different initial compositions. Vertical dashed lines indicate the time of progression of CT (yellow) and AT (blue), respectively. B) Gain in TTP by AT compared to CT as a function of initial proximity to K and abundance of resistance. C) dR/dt as a function of S and R, together with treatment trajectories for Tumours 1 ($(n_0, f_R) = (25\%, 0.1\%)$) & 4 ($(n_0, f_R) = (75\%, 0.1\%)$) from A. Crosses indicate progression. This shows how AT extends TTP by minimising dR/dt via competition, and demonstrates that for certain tumours AT can extend TTP even if no cost of resistance is present.*

tance of the presence of a cost of resistance in determining whether CT or AT will achieve a longer TTP. We will model the cost as a decrease in the resistant cell growth rate, r_R, which is the how it has been modelled most commonly in the past (e.g. [20, 17, 22]). We will determine TTP according to the RECIST criteria as a 20% increase in tumour size from the pre-treatment baseline, where TTP_{AT} and TTP_{CT} will refer to the TTP under AT and CT, respectively.

4.1 Adaptive therapy does not *per se* require a cost of resistance

In order to develop a baseline understanding of when and how AT can extend TTP in our model, we compare CT and AT under the assumption that both turnover ($\hat{d}_T = 0$) and the cost of resistance can be neglected ($\hat{r}_R = 1$). Since AT seeks to control the expansion of drug-resistant cancer cells through competition, we hypothesise that both the abundance of resistance (f_R) and the proximity to carrying capacity (n_0), which reflects the intensity of resource competition, are important determinants of the benefit of AT over CT. To test this hypothesis, we simulate treatment under each approach for 9 different tumours representing three levels of *a priori* resistance and three different levels of proximity to carrying capacity. We find that that the benefit of AT is greatest when there is little resistance ($f_R = 0.1\%$) and the tumour is close to K ($n_0 = 75\%$; $\text{TTP}_{AT} = 146.8\%$ TTP_{CT}; Figure 3A). To further characterise this relationship, we extend our analysis to 18 tumours and plot the gain in TTP under AT as a function of the tumour composition parameters in Figure 3B. We find that if the abundance of resistance is high, AT provides no benefit over CT, independent of how close the lesion is to its carrying capacity (Figure 3B). In contrast, if resistance is low, AT prolongs the TTP in a non-linear manner dependent on both the abundance of resistance and the ratio n_0. We conclude that AT can improve TTP, even in the absence of a cost, and that this gain is greatest the closer the tumour is to carrying capacity and the lower the initial abundance of resistance.

4.2 Treatment vacations provide a benefit only if intratumoural competition is strong

Moreover, we find that each of the two characteristics of the tumour's initial composition has a distinct impact on the treatment dynamics. As we increase the initial abundance of resistance from 0.1% to 10%, we decrease the number of completed AT cycles (Figure 3A). In the most extreme case, at 10% initial resistance, treatment can not decrease the tumour burden sufficiently to trigger any treatment withdrawal in the three tumours. In contrast, increasing the level of growth saturation in a tumour (proximity to carrying capacity) does not alter the AT cycle number, but does increase the benefit delivered by each cycle. For example, even though all tumours with 1% initial resistance complete one AT cycle, a meaningful benefit in TTP is only achieved in the case when the tumour is 75% saturated (Figure 3A). The reason for this is that the competition exerted

by sensitive cells only has significant impact on the growth of the resistant cells if the tumour is close to K. In summary, for AT to provide a benefit in practice, we require not only competition, but also that the tumour burden can be decreased sufficiently to allow for treatment withdrawal.

4.3 AT extends TTP by minimising dR/dt

How can we explain the benefit of AT over CT in the absence of a cost of resistance? Hansen et al [24] present a framework for deciding how to best control a drug-resistant pathogen. Extending their line of argument, we can derive the following explanation: Firstly, we note that if the sensitive population becomes too large, we can decrease its size by treatment. Thus, in our model it is primarily the rate of expansion of the resistant population, dR/dt, which determines TTP. The faster the expansion, the shorter the TTP, and vice versa. To illustrate this, in Figure 3C we plot $dr/d\tau$ (which is a re-scaled form of dR/dt) as a function of s and r. This shows an area of maximal resistant growth rate centred at $(s, r) = (0, \frac{1}{2}(1 - \frac{\hat{d}_T}{\hat{r}_R})) = (0, 0.5)$, and illustrates how dR/dt is smaller, the lower the abundance of resistance, and the closer the tumour is to carrying capacity. In addition, we plot the treatment trajectories for Tumour 1 and 4 from Figure 3A. We see that both trajectories for Tumour 1 and the CT trajectory for Tumour 4 run through the area of maximum $dr/d\tau$ (Figure 3C). In contrast, the trajectory for Tumour 4 under AT bypasses this region. This illustrates how AT trades-off an increased tumour burden for a slower expansion of resistance.

We can quantify this argument and clarify the role of each parameter by considering the equation for dR/dt (Equation (2)). This may be rewritten as:

$$\frac{dR}{dt} = \underbrace{\left(r_R - d_R - \frac{r_R R}{K}\right) R}_{\star \text{ Local Expansion}} \quad - \quad \underbrace{\frac{r_R S}{K} R}_{\dagger \text{ Control via Competition}} \quad . \tag{8}$$

Treatment under CT very rapidly removes the sensitive cells, effectively eliminating the negative term representing competition (†) in Equation (8). In contrast, AT maintains sensitive cells at some non-zero level, S_{AT}, for some time $t \in [0, t^*]$. Assuming CT eradicates sensitive cells very quickly so that their contribution to recurrence under CT can be neglected, we approximately have for $t \in [0, t^*]$:

$$\begin{aligned} \left.\frac{dR}{dt}\right|_{\text{CT}} &\approx \left(r_R - d_R - \frac{r_R R}{K}\right) R \\ &\geq \left(r_R - d_R - \frac{r_R R}{K}\right) R - \frac{r_R S_{AT}}{K} R \approx \left.\frac{dR}{dt}\right|_{\text{AT}}, \end{aligned}$$

where $\left.\frac{dR}{dt}\right|_{\text{CT}}$ and $\left.\frac{dR}{dt}\right|_{\text{AT}}$ denote the growth rate of the resistant population under CT treatment and AT, respectively. We can see that through the term representing competition, AT reduces dR/dt. This is why it can provide benefits

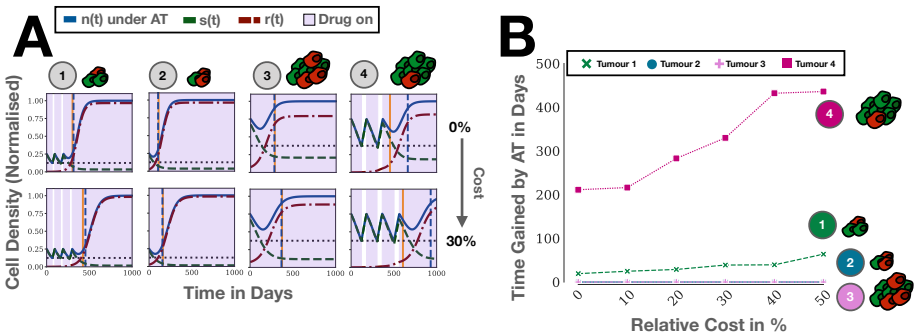


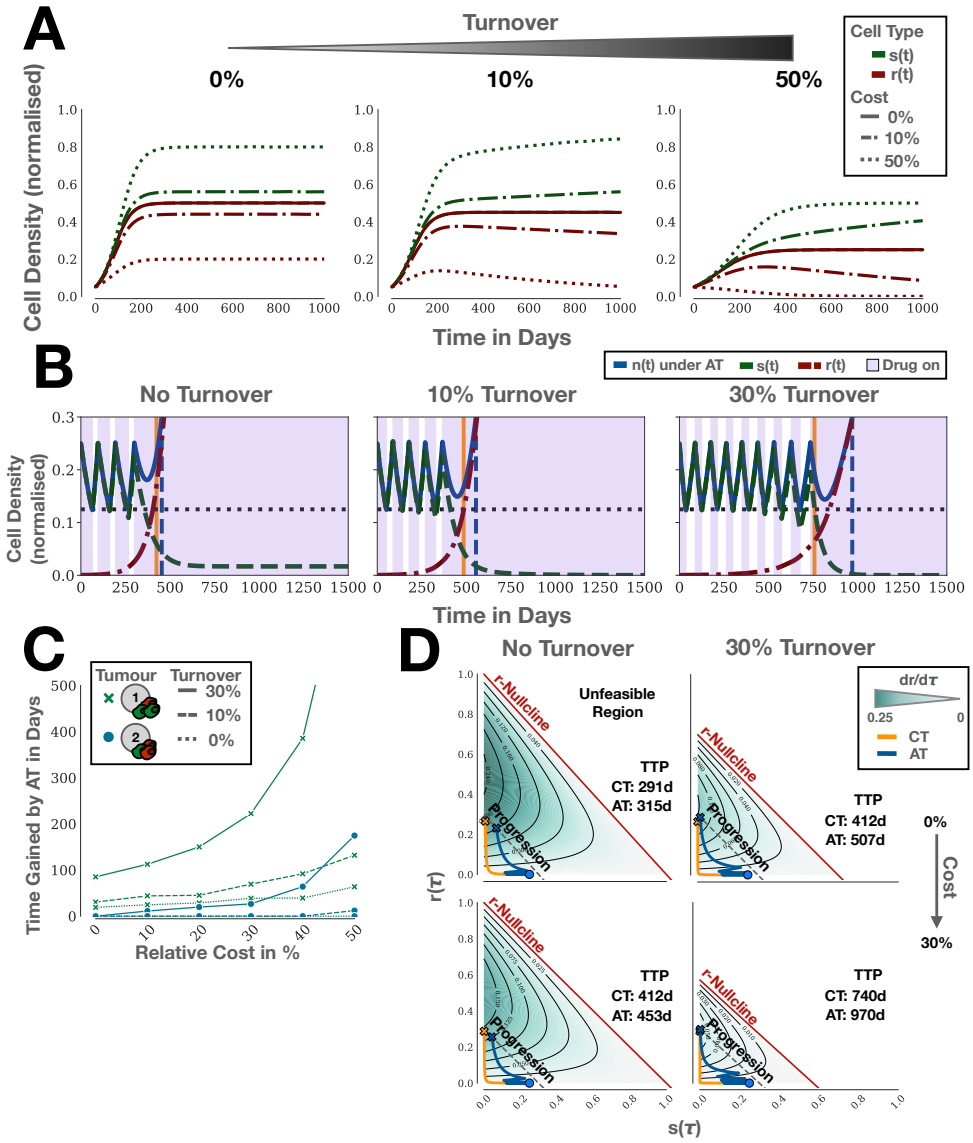
Figure 4: *In the absence of turnover ($\hat{d}_T = 0$) a cost of resistance enhances AT only when resistance is rare and tumours are close to carrying capacity. E) Simulations of Tumours 1-4 from Figure 3A with and without a 30% cost of resistance ($\hat{r}_R = 70\%$; Tumours 1 & 4 given in Figure 3; Tumour 2: $(n_0, f_R) = (25\%, 10\%)$; Tumour 3: $(n_0, f_R) = (25\%, 10\%)$). F) Gain in TTP by AT compared to CT for different levels of cost of resistance for Tumours 1-4.*

even in the absence of a cost of resistance, and why AT is most effective when the tumour is close to local carrying capacity. In addition, this implies that the most effective AT would maintain the tumour at its original size for as long as possible in order to maximise the effect of competition.

4.4 In the absence of turnover, a cost of resistance does not increase the time gained by AT

Next, we examine whether a cost of resistance enhances the benefit of AT. We still assume that no turnover is present but now assume a 30% decrease in proliferation rate of the resistant population ($\hat{r}_R = 70\%$) and simulate CT and AT for the four corner cases from Figure 3A. As expected, TTP_{AT} increases in all four cases (Figure 4A; compare vertical blue dashed line in top and bottom row). However, the effect is much more pronounced when the tumour is close to carrying capacity and the initial fraction of resistance is small (Tumour 4; Figure 4A). Moreover, TTP_{CT} increases as well, so that significant gains compared to CT are only seen for Tumour 4 (difference in TTP extended from 211d to 330d; 4A). To further quantify this observation we compute the difference between TTP_{AT} and TTP_{CT} for each of the four tumours in Figure 4A for costs ranging from 0% to 50%. This shows that in the absence of turnover, a cost of resistance significantly increases the benefit of AT only for Tumour 4, that is only if resistance is rare and the tumour is close to carrying capacity (Figure 4A).

4.5 Turnover mediates the impact of a cost of resistance


A
Turnover
0%
10%
50%
Cell Type
s(t)
r(t)
Cost
0%
10%
50%
Cell Density (normalised)
Time in Days
B
n(t) under AT
s(t)
r(t)
Drug on
No Turnover
10% Turnover
30% Turnover
Cell Density (normalised)
Time in Days
C
Tumour
Turnover
30%
10%
0%
Time Gained by AT in Days
Relative Cost in %
D
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CT
AT
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CT: 291d
AT: 315d
TTP
CT: 412d
AT: 507d
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CT: 412d
AT: 453d
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CT: 740d
AT: 970d
Progression
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r(τ)
s(τ)

Figure 5 *(previous page)*: *Turnover is an important determinant of the success of AT. A: Simulations of our model in the absence drug, illustrating the impact of a cost of resistance and of turnover. In the absence of turnover, even a significant cost does not result in the elimination of the resistant cells. In contrast, if we increase the natural death rate, resistant cells paying a cost are more and more quickly outcompeted by the sensitive cells. B: Simulations of AT for Tumour 1 from Figure 4A with a 30% cost of resistance and increasing levels of turnover. While in the absence of turnover there is little benefit to AT, despite a 30% cost of resistance, AT can obtain significant gains in TTP in the presence of turnover. C: Relationship between the cost of resistance and the difference between* TTP_{AT} *and* TTP_{CT} *for Tumours 1 and 2 for increasing levels of turnover. The larger the turnover, the larger the gain through AT and the greater the impact of a cost. D:* dR/dt *as a function of* s *and* r *with and without cost and turnover. Overlaid on top are the treatment trajectories for Tumour 1 under AT and CT. The closer the tumour (blue dot) initially is to the* r*-nullcline (red line), the longer AT can reduce* dR/dt *and prevent progression. In the absence of turnover, the position of the* r*-nullcline depends solely on the carrying capacity. In its presence, it is mediated by the rate of turnover, and as such also the cost of resistance. Thus, in order to understand the impact of a cost we also have to consider the turnover in the tumour.*

So far we have assumed that turnover of tumour cells is negligible ($\hat{d}_T = 0$). However, tumours are subject to resource starvation and immune predation, resulting in continuous tumour turnover. In Figure 5A we show how such turnover modulates the impact of a cost of resistance on the competition between sensitive and resistant cells in the absence of drug. In the absence of turnover, both populations grow until they make up a fixed proportion of the tumour given in the limit by $(S, R) = (S^*, K - S^*)$, where S^* satisfies the equation $R_0 \left(S^*/S_0\right)^{r_R/r_S} + S^* = K$ (Figure 5A). Importantly, while a cost of resistance reduces the number of resistant cells, these cells never go extinct (Figure 5A). In contrast, if we introduce natural turnover ($\hat{d}_T > 0$), resistant cells go extinct over time (Figure 5A). We conclude that the rate of extinction depends on both the cost of resistance and the rate of turnover.

Based on this observation, we hypothesise that it is important to consider the rate of turnover in the tumour when assessing its response to AT. To test this hypothesis, we repeat our analysis of Tumour 1 from Figure 4A under different rates of turnover (Figure 5B). In the absence of turnover, AT provides little benefit despite a 30% cost of resistance (Time Gained: 30d). In contrast, with 10% turnover ($\hat{d}_T = 10\%$) AT extends TTP by 69d, and at 30% turnover ($\hat{d}_T = 30\%$) this increases to 212d. To further quantify this, we compute the relationship between the relative TTP gained by AT and the cost of resistance for different levels of turnover for Tumours 1 and 2 from Figure 3A. This shows that the higher the turnover, the greater the relative time gained by AT, and the more AT is enhanced by a cost of resistance (Figure 5C). For example, in the absence of turnover, AT can not extend TTP for Tumour 2 even when there is a 50% cost of resistance. However, if the turnover is 10% then the relative

time gained by AT increases monotonically with the cost, and reaches gains of up to 175d. We conclude that turnover is a key determinant of the extent to which AT can exploit a cost of resistance.

4.6 Turnover improves AT even in the absence of a cost of resistance

Furthermore, Figure 5C shows that turnover alters the dynamics not only in the presence, but also in the absence, of a cost. Without turnover, AT extends TTP by only 20d compared to CT for Tumour 1. If we assume a 30% turnover, this increases to 112d (Figure 5C). This implies that AT may be beneficial in a wider range of tumours than might be expected from Figure 4B. While greater proximity to carrying capacity and a smaller initial fraction of resistance increase the benefit derived from AT and from a cost, Figure 5C shows that in the presence of turnover even tumours far away from K and with substantial initial fractions of resistance can derive a benefit from AT.

4.7 AT is driven by proximity to the population equilibrium, not carrying capacity

Why does turnover increase the benefit of AT even in the absence of a cost of resistance? As we illustrate in Figure 3C, AT works by reducing dR/dt over time. The smallest value of dR/dt is reached along the R-nullcline along which $dR/dt = 0$. This line is plotted in red in Figure 3C and is given in fully parametrised form by the equation:

$$R(t) = \left(1 - \frac{d_T}{r_R}\right) K - S(t). \tag{9}$$

Biologically speaking, this line describes the population equilibrium of the resistant population for a given number of sensitive cells, that is, the number of resistant cells at which growth saturates. The closer a tumour is to this line, the slower the expansion of the resistant population. This is why in our model tumours which are initially closer to carrying capacity can be controlled better by AT than tumours far away from it (compare Tumours 1 & 4 in Figure 3C). Moreover, Equation (9) shows that the larger $\frac{d_T}{r_R}$, the smaller the number of sensitive cells required to control the growth of resistant cells through competition. Mathematically speaking, larger turnover corresponds to a shift of the R-nullcline downwards. We illustrate this in Figure 5D. As we increase the turnover from 0% to 30%, the R-nullcline is translated downwards, and the overall growth rate of the resistant population decreases. Moreover, Equation (9) shows that a cost of resistance has an effect on the position of the R population equilibrium if and only if $d_T \neq 0$. This is illustrated in Figure 5D, where a cost of resistance only results in a change in the distance between the initial tumour and the population equilibrium if turnover is present. To sum up, the success of AT in general depends not on the proximity to carrying capacity but

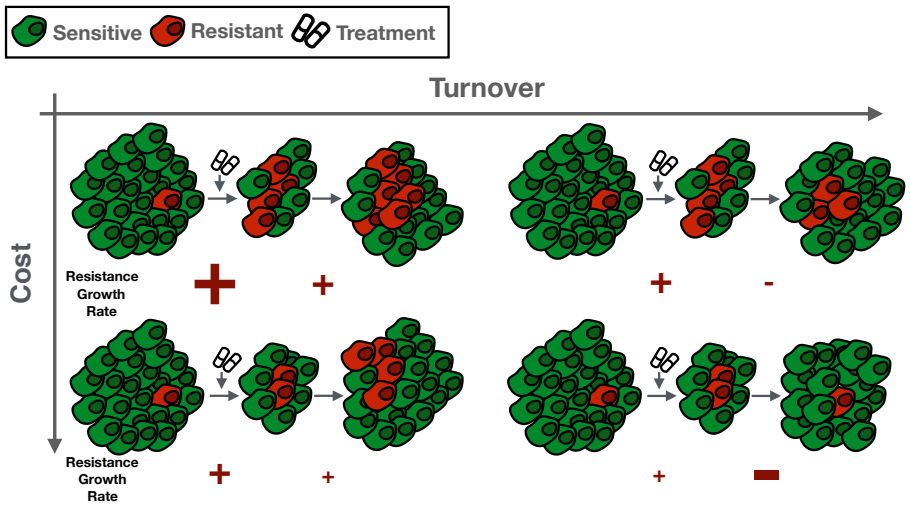

Figure 6: *The interplay between cost of resistance and turnover in AT. In the absence of both, the resistant population expands rapidly under treatment (large positive resistance growth rate). While drug withdrawal can slow down further expansion, it can not reverse it (moderate growth rate). A cost of resistance slows down the emergence of resistance, but without turnover, it can also not revert it (small positive growth rates). In contrast, the presence of turnover results in a selection pressure which can reduce the frequency of resistance in the off-periods, especially if a cost of resistance is present (negative resistance growth rates).*

on how close the resistant population is to its population equilibrium. If the death rate is negligible, then this is indeed the carrying capacity. However, in the presence of a background death rate, turnover has to be taken into account and the equilibrium can be far from the environmental carrying capacity.

5 Discussion and Conclusion

With four clinical trials of AT already ongoing (clinicaltrials.gov identifiers: NCT02415621; NCT03511196; NCT03543969; NCT03630120) and more in preparation, it is important to develop criteria to identify which patients might benefit from AT. Intuitively, it appears that the presence of a fitness cost of resistance would be a great facilitator of AT, and should be considered as an inclusion criterion. However, as Bacevic et al [3] and Gallaher et al [17] show, the presence of a cost does not guarantee that the resistant population can be effectively controlled. Moreover, the experimental work which motivated the trial in prostate cancer did not find a cost of resistance *in vitro*, yet AT has so far extended median TTP by over 27 months in prostate cancer patients [47].

The aim of this paper is to consolidate these findings and develop an under-

standing of the circumstances under which the presence of a cost of resistance is required for AT to provide a benefit, and under which circumstances it is not. To do so, we developed a simple two-population Lotka-Volterra ODE model and treated it according to the AT algorithm from [47]. As would be expected, we find that the larger the cost of resistance, the longer TTP under AT. However, we show that whether any, and if so how much, time is gained compared to CT depends on three key aspects: i) the initial fraction of resistant cells (f_R), ii) the proximity of the tumour to environmental carrying capacity (n_0), and iii) the rate of cell turnover (d_T). Previously it has been shown that the smaller the fraction of resistance in a tumour and the closer it is to carrying capacity, the greater the benefit of AT [3, 24, 17]. We extend these findings by showing that for such tumours a cost of resistance is not necessary for AT to be beneficial. While its presence increases the time gained by AT, our work suggests that significant benefits may be obtained even in its absence. Working in parallel, Viossat and Noble (*in preparation*) have reached similar conclusions, yet our analyses extend beyond theirs in discussing in detail the impact of a cost on the treatment dynamics and highlighting the key role of cellular turnover.

Since AT aims to exploit competition between cells it makes sense that its efficacy increases the closer the tumour is to carrying capacity. However, what is the meaning of carrying capacity in a dynamic environment such as a tumour, and can AT benefit patients whose tumours are far away from carrying capacity? Notably, we show that for such patients it is important to consider both the cost of resistance as well as the turnover in the tissue (see Figure 6 for a summary). For example, even though Tumour 1 is far away from carrying capacity, significant gains in TTP may be achieved with AT if the turnover in the tumour is sufficiently high (Figure 5C). The reason for this is that the shorter the life span of a cell, the fewer opportunities it will have to divide, and the greater will be the impact of interference through competition. As previously discussed in the ecology literature (see [33] for an in-depth review), this fact is easily overlooked when working with the most commonly taught form of the logistic growth or the Lotka-Volterra equations, but is important to keep in mind when studying competition. A concern which has been raised about the feasibility of AT in practice is that the tumour is required to be very close to carrying capacity [23]. We propose that if turnover is taken into account, a tumour does not have to be as close to its carrying capacity as may be otherwise expected.

Moreover, as we illustrate in Figure 5A, turnover modulates the intensity of selection against resistance during the drug-free intervals. If cell death is negligible, then resistant cells can 'hide out' for long times until drug is re-applied, even if there is a cost of resistance. If there is turnover, then this is not possible. Thus, the impact of a cost of resistance is modulated by turnover in the tumour. If the turnover is high, then a lot of time may be gained. If the turnover is low, then a cost of resistance has less of an impact and TTP is primarily determined by the tumour's proximity to carrying capacity and the initial fraction of resistance.

What is the turnover rate of tumour cells? While this is challenging to

quantify in patients, the existing evidence paints a very dynamic picture of growing tumours. The presence of substantial areas of necrosis in many tumours and the significant discrepancy between the doubling time expected from the fraction of dividing cells seen in histological analyses and the actually observed doubling time, indicate substantial cell loss [40, 41, 31, 29]. Although some of this loss may be attributed to emigration of cells from the tumour and the fact that physical compression might reduce the volumetric doubling time, it is likely that turnover in tumours exceeds 50% [40, 41, 31]. It is also important to remember that in homeostatic tissue the turnover rate is 100%, as the tissue continuously renews itself without changing in size [31]. Thus, the range of values considered in this paper is probably a very conservative estimate and the true impact may be larger than indicated here. Going forward it will be important to obtain more accurate estimates of the tumour cell turnover (many of the aforementioned estimates were obtained in the 1970s and 80s), and assess its role in tumour drug response, specifically in relation to adaptive therapy.

There are a number of simplifications we make in our model. Firstly, similar to [3, 24] we assume that the tumour is not curable. However, as can be seen in Figure 5D a cost of resistance and greater turnover also result in fewer cells at the Nadir during CT. This implies that the tumours in which AT will be most effective are those in which CT just misses being curative, an observation also made by Gallaher et al [17]. Thus, the very long gains predicted under AT have to be viewed with some scepticism as in a proportion of these patients CT would have cured the tumour. That being said, AT is intended for advanced settings in which the available treatment is rarely curative, which suggests that such cases will be rare [19, 12]. Moreover, it seems plausible that if one finds that AT controls the tumour very well, one could still decide to switch to a curative approach. In fact, AT has been shown to have stabilising effects on tumour vasculature [14], which suggests that a curative approach may be even more effective after an initial period of AT.

Furthermore, we do not consider the potential health-risks associated with the increased tumour burden under AT. As a result, our model predicts that the longest TTP can be achieved by keeping the tumour as close to its initial size as possible. This is true also for most previous studies carried out in this area ([20, 3, 17, 39]). As Hansen et al [24] show, if one accounts for potential *de novo* resistance acquisition this can generate situations in which maintaining a pool of sensitive cells can in fact accelerate the growth of the resistant population. We propose that investigating the risks associated with the maintained tumour burden under AT should be an important area of future research.

Finally, in order to facilitate analysis we neglect the impact of space in this manuscript. However, both [3] and [17] have previously demonstrated that in the absence of turnover the spatial architecture of the tumour is a key determinant of the success of AT. If resistant cells can be "trapped" by sensitive cells in the centre of the tumour, the tumour may be controlled for a long time [3, 17]. Going forward it would be important to assess whether our conclusions extend to a spatial setting. While turnover will increase the selection against resistant cells, it might also facilitate their escape by creating gaps in the layer of sensitive

cells surrounding them. Interestingly, using an extension of their model in [17] Gallaher et al [16] have already shown that turnover mediates the proliferation-migration trade-off in a tumour. As such, an important next step would be to extend our analysis to a spatial model of AT.

To conclude, we want to point out interesting parallels and possible opportunities from the pest and antibiotic resistance management literature, which have investigated fitness costs of resistance since the mid-20th century (see, for example, [11] for an early review of cost of resistance in insectides). Firstly, we note that similar to cancer it has always been a controversial issue. For example, Bergelson et al [6], find that out of 88 studies examining the cost question in plants, 44 show a cost, 40 find no difference and 4 even observe a benefit to resistance. Similar patterns can be seen for pesticide resistance [11, 18] and antibiotic resistance [2, 1]. Whether a cost is present depends on the resistance mechanism, and its environmental as well as genetic context [6, 1, 30]. Thus, in developing AT we should not assume that a cost of resistance is always present or is uniform throughout the tumour, or that it will persist throughout treatment. That being said, in line with the predictions of this paper, resistance management can be successful even if the cost of resistance is uncertain or absent. For example, the resistance management scheme for the insecticide producing *Bacillus thuringensis* (Bt) crop has been successful despite mixed results regarding the presence of cost of resistance [42, 18, 7]. Two solutions have been developed for pursuing resistance management despite uncertainty about a cost of resistance. Firstly, [7] proposed a dynamic framework which estimates the cost of resistance from the data received from initial treatment and updates the strategy as treatment proceeds. It could be interesting to apply this scheme to cancer. Secondly, many pest resistance management strategies include multiple treatment modalities to increase robustness of the scheme [36, 8]. Some initial work on multi-drug AT has already been carried out [46, 45, 12]. Going forward it will be important to extend this work to develop strategies which exploit a cost of resistance if and for as long as it is present, yet are robust to when this cost disappears due to environmental or genetic compensation.

Acknowledgements

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A1 Appendix

A1.1 Steady State Analysis

For completeness we here analyse the more general model discussed in Section A1.2 of which the model which forms the focus of the main paper is a special case. We will begin with a general discussion before providing two specific examples illustrating the key features of the phase plane dynamics which are driving our results. Assuming CT at dose $D(t) = D^*$ Equations (10)-(11) have the following steady states:

- Tumour Elimination (SS1): $(S^*, R^*) = (0, 0)$. A linear stability analysis gives eigenvalues $\lambda_1 = r_S(1 - d_D D^*) - d_S$ and $\lambda_2 = r_R - d_R > 0$, which shows that this state is always unstable and so, unless there are no resistant cells in the tumour or $d_R > r_R$, tumour elimination is not possible in this model. This is because there is no mechanism in this model to prevent the outgrowth of resistant cells except for inhibition from sensitive cells.

- A Fully Sensitive Tumour (SS2): $(S^*, R^*) = \left(\left(1 - \frac{d_S}{r_S(1-d_D D^*)}\right) K_S,\, 0\right)$ which corresponds to a tumour consisting entirely of sensitive cells. This state has eigenvalues $\lambda_1 = r_R - d_R - r_R \left(1 - \frac{d_S}{r_S(1-d_D D^*)}\right) \frac{K_S}{K_R}$ and $\lambda_2 = r_S(d_D D^* - 1) \left(1 - \frac{d_S}{r_S(1-d_D D^*)}\right)$, so that its feasibility and stability depend on the effective drug kill rate $d_D D^*$, and the ability of the resistant cells to invade the equilibrium, which is determined by the balance of terms expressed in λ_1.

- A Fully Resistant Tumour (SS3): $(S^*, R^*) = \left(0,\, \left(1 - \frac{d_R}{r_R}\right) K_R\right)$ which describes a tumour consisting entirely of drug resistant cells. This state has eigenvalues $\lambda_1 = r_S(1 - d_D D^*) \left(1 + \left(\frac{d_R}{r_R} - 1\right) \frac{K_R}{K_S}\right) - d_S$ and $\lambda_2 = d_R - r_R < 0$, which implies its stability is dependent on the sign of λ_1.

- Coexistence (SS4): $(S^*, R^*) = \left(S,\, \left(1 - \frac{d_R}{r_R}\right) K_R - S\right)$, which describes a set of tumours consisting of a mixture of sensitive and resistant cells. This set only exists if the two non-zero nullclines precisely overlap (see Figure A1A for an illustration). No simple analytical form is available for the corresponding eigenvalues, and thus, they will be omitted here.

Having discussed the eigenvalues in general we will briefly show four specific examples to illustrate the impact of the turnover and a cost of resistance on the phase plane dynamics. To do so, we will again adopt the simplifying assumptions from Section 3.1 that $d_S = d_R := d_T$ and $K_S = K_R := K$. Unless otherwise stated simulations were done with the parameters from Section 3.2.

A1.1.1 No turnover

Assuming $d_T = 0$, we obtain the eigenvalues shown in Table A1. We note that r_R has no effect on the position of the steady states and merely scales the

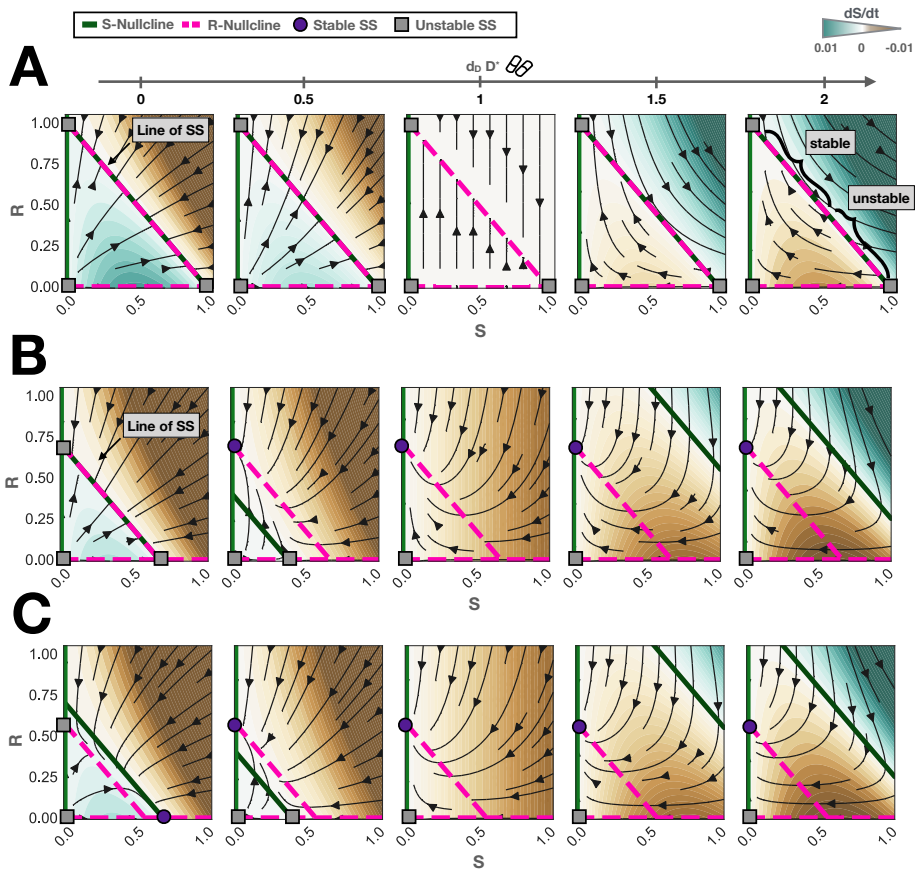

Figure A1: *Phase planes of the model (Equations* (5) *and* (6)*) under CT with increasing levels of drug kill,* $d_D D^*$ *(parameters as in Table 1). We assume no cost of resistance* ($r_R = r_S$)*. SS stands for steady state. A) No turnover and no cost* ($d_T = 0$, $r_R = r_S$)*. There is a continuum of steady states (SS4) given by* $S + R = 1$*. For* $d_D D^* < 1$ *the tumour continues to grow until it reaches carrying capacity on SS4. For* $d_D D^* > 1$ *sections of SS4 become unstable and we see the formation of heteroclinic orbits between unstable and the stable section of SS4. This represents tumours which are largely sensitive so that they will initially experience tumour shrinkage, but will subsequently recur due to the outgrowth of resistance. B) Turnover but no cost* ($d_T = 0.1$, $r_R = r_S$)*. Unless* $d_D D^* = 0$*, the tumour becomes fully resistant over time. C) Turnover and cost* ($d_T = 0.3$, $r_R = 0.7$)*. Provided the tumour is treated at low dose* ($d_D D^* < 1 - r_S/r_R$)*, it can be maintained fully sensitive and decreased in size. If the dose is escalated beyond this threshold, the tumour will evolve to become fully resistant.*

State	Description	A) No Turnover ($d_T = 0$)	B) With Turnover ($0 < d_T < r_S, r_R$)
SS1	*Tumour Elimination*	$\lambda_1 = \mathbf{r_S(1 - d_D D^*)}$ $\lambda_2 = r_R$	$\lambda_1 = \mathbf{r_S(1 - d_D D^*) - d_T}$ $\lambda_2 = r_R - d_T$
SS2	*Sensitive Tumour*	$\lambda_1 = 0$ $\lambda_2 = \mathbf{r_S(d_D D^* - 1)}$	$\lambda_1 = \mathbf{d_T\left(\frac{r_R}{r_S(1-d_D D^*)} - 1\right)}$ $\lambda_2 = \mathbf{r_S(d_D D^* - 1)\left(1 - \frac{d_T}{r_S(1-d_D D^*)}\right)}$
SS3	*Resistant Tumour*	$\lambda_1 = 0$ $\lambda_2 = \mathbf{-r_R}$	$\lambda_1 = \mathbf{d_T\left(\frac{r_S}{r_R}(1 - d_D D^*) - 1\right)}$ $\lambda_2 = \mathbf{d_T - r_R}$
SS4	*Coexistence*	No simple form available	No simple form available

Table A1: *Eigenvalues for the cases considered in Figure A1 (assuming $d_D D^* \neq 1$).* ***Bolded values*** *indicate eigenvalues which are negative in at least part of the parameter space.*

eigenvalues so that a cost of resistance will have no influence on the structure of the phase space in this case. Thus, to simplify the following analysis we will assume there is no cost ($r_R = r_S$).

Both SS2 and SS3 have zero eigenvalues because they lie on the manifold of steady states given by SS4. Solving numerically for the phase plane we find that between $d_D D^* = 0$ and $d_D D^* = 1$, the tumour will keep growing until it reaches a point on SS4 and saturates (Figure A1A). The greater the value of $d_D D^*$, the larger the proportion of resistant cells at equilibrium. When $d_D D^* = 1$, dS/dt becomes exactly 0 everywhere (Figure A1A; see also Equation 1). As $d_D D^*$ is increased above 1, the sign of dS/dt switches (Figure A1A). Moreover, parts of the line SS4 become unstable, and we observe the formation of heteroclinic orbits connecting the unstable portions of SS4 to the stable portions of SS4 (Figure A1A). Biologically, the transition through $d_D D^* = 1$ represents a transition from when the drug only reduces the net growth of the population to when it actually causes decreases in the population size. The heteroclinic orbits represent tumours which initially shrink in size but subsequently recur. Interestingly, the fully resistant state appears to become the globally absorbing state only as $d_D D^* \to \infty$. This is because the resistant cells protect the sensitive cell by saturating the environment, thereby preventing them to divide which makes them no longer sensitive to the drug.

A1.1.2 With turnover

If we assume that turnover is present ($0 < d_T < r_S, r_R$) we obtain the eigenvalues given in the right-hand side column of Table 1. To begin with, let us assume that there is no cost of resistance ($r_R = r_S$). If $d_D D^* = 0$, then we again have a line of steady states, but this time the tumour saturates below the carrying capacity (Figure A1B). Importantly, if the drug kill is increased, then the S-nullcline given in dimensional form by $S(t) = \left(1 - \frac{d_T}{(1-d_D D^*)r_S}\right) K - R$, is now translated downwards. As a result, for $0 < d_D D^* < 1$, SS4 disappears and

SS3 becomes stable ($\lambda_1, \lambda_2 < 0$) whereas SS2 is unstable ($\lambda_1 > 0$; see also Figure A1B). Thus, in contrast to when turnover is absent, in the presence of turnover the tumour evolves to become fully resistant even if treated at a very low dose (Figure A1B). When $d_D D^*$ is increased above 1, SS2 takes on a value above K which converges to K from above as $d_D D^* \to \infty$ (see the expression for SS2 in Section A1.1 and Figure A1B). Whilst positive, this state is not biologically realistic as it implies that the population can grow beyond the environmental carrying capacity.

Finally, we consider the impact of a cost of resistance in this setting ($r_R < r_S$). From Table A1 we can see that provided $d_D D^* < 1 - r_S/r_R$, SS2 is stable whereas SS3 is unstable. Thus, in the presence of a cost low dose treatment can shrink the tumour whilst maintaining it stably sensitive (Figure A1C). However, if the drug kill is intensified beyond $1 - r_S/r_R$ SS2 becomes unstable and SS3 becomes stable (Table A1) and the tumour will become fully resistant over time (Figure A1C).

A1.2 Extension to other types of cost

So far we have assumed that the cost of resistance manifests itself in the proliferation rate of the cells. However, it is plausible that it might manifests itself in the carrying capacity or the death rate. Carrere et al [10], for example, report that Epothilene resistant lung cancer cells are larger than their sensitive parental cells so that their carrying capacity is reduced. To study the dynamics of such manifestations of a cost we will extend our model to the following more general form:

$$\frac{dS}{dt} = r_S \left(1 - \frac{S+R}{K_S}\right)(1 - d_D D(t))\, S - d_S S, \tag{10}$$

$$\frac{dR}{dt} = r_R \left(1 - \frac{R+S}{K_R}\right) R - d_R R, \tag{11}$$

$$N(t) = S(t) + R(t), \tag{12}$$

As we have shown that the R-nullcline provides a useful framework for understanding the impact of a cost in the growth rate, we will begin by considering how this extends to costs in the carrying capacity and the death rate. The R-nullcline of this more general system is given by:

$$R(t) = \left(1 - \frac{d_R}{r_R}\right) K_R - S(t). \tag{13}$$

From Equation (13) we can see that changes in K_R and d_R can also be understood as reducing the resistant population equilibrium level and translating the R-nullcline along the R-axis. That being said, the magnitude of the change caused by a relative cost, $c \in (0,1)$, differs depending on which parameters the cost is assumed to manifest itself in. Specifically:

Theorem A1.1. *Let* $R_r^* = \left(1 - \frac{d_R}{(1-c)r_R}\right) K_R$, $R_d^* = \left(1 - \frac{(1+c)d_R}{r_R}\right) K_R$, *and* $R_K^* = \left(1 - \frac{d_R}{r_R}\right)(1-c)K_R$ *denote the population equilibria under a relative cost of* c (> 0) *in* r_R, d_R, *and* K_R, *respectively. Then:*

1. *The impact of a cost in* r_R *will always be greater than the impact of a cost in* d_R. *That is,* $R_r^* > R_d^*, \forall c \in (0,1)$.

2. *Provided* $d_R < \frac{c}{1+c} r_R$, *the impact of a cost in* K *will be greater than the impact of a cost in* d_R $(R_K^* > R_r^*)$. *Otherwise a cost in* d_R *will have a greater impact.*

3. *The impact of a cost in* K *will be greater than the impact of a cost in* r_R $(R_K^* > R_r^*)$ *provided* $d_R < \frac{1-c}{2-c} r_R$. *Otherwise a cost in* r_R *will have a greater impact.*

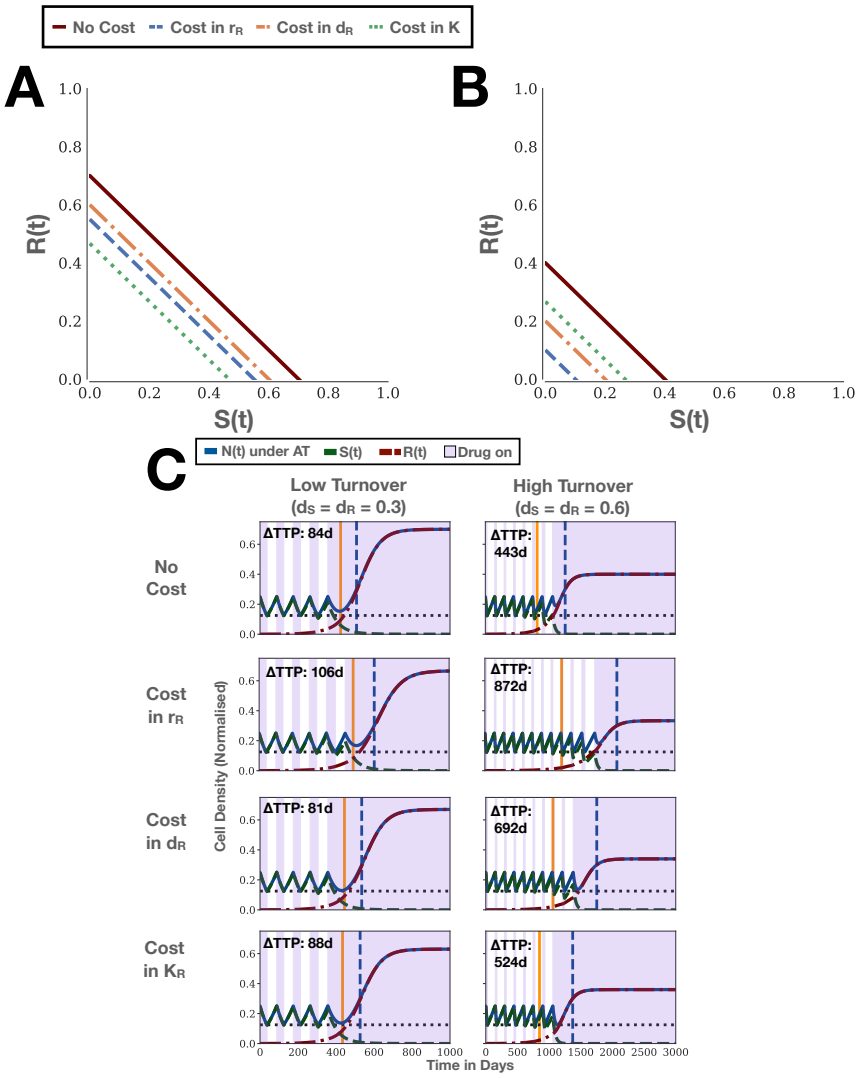

Figure A2: *Comparison of the impact of different manifestations of the cost of resistance. A) Comparison of the impact of a 30% cost in r_R, d_R and K_R on the R-nullcline with small turnover ($d_R = 0.3r_R$). All manifestations of a cost can be thought of as a vertical shift of the R-nullcline with a cost in K_R achieving the largest shift in this parameter regime. B) Comparison of the impact of a 30% cost in r_R, d_R and K on the R-nullcline with high turnover ($d_R = 0.6r_R$). Again, all types of cost shift the R-nullcline but this time the costs in r_R and d_R have a greater effect than the cost in K_R. This illustrates how the impact of a cost, independent of how it manifests itself, depends on its context (here given by the balance of r_R, d_R and K_R). C) Treatment dynamics under AT for Tumour 1 from Figure 3 ($(n_0, f_R) = (0.25, 0.999)$) without a cost and with a 10% cost in each of r_R, d_R and K_R. This shows that a cost in r_R is most effective in prolonging TTP under AT, as it is able to reduce the growth rate of the resistant population when it is small and so can keep it small for longer. Note, in the simulations of a cost in death rate, d_R does not take the values shown, but values 10% greater.*

Proof. Claim 1: Comparing R_r^* and R_d^* we find:

$$\begin{aligned}
&R_r^* > R_d^* \\
\leftrightarrow &\left(1 - \frac{d_R}{(1-c)r_R}\right) K_R > \left(1 - \frac{(1+c)d_R}{r_R}\right) K_R \\
\leftrightarrow &(1+c) < \frac{1}{1-c} \\
\leftrightarrow &c^2 > 0,
\end{aligned}$$

so that $R_r^* > R_d^*, \forall c \in (0,1)$.

Claim 2: Similarly, comparing R_K^* and R_d^* gives:

$$\begin{aligned}
&R_K^* > R_d^* \\
\leftrightarrow &\left(1 - \frac{d_R}{r_R}\right)(1-c)K_R > \left(1 - \frac{(1+c)d_R}{r_R}\right) K_R \\
\leftrightarrow &-c(1+c)d_R - r_R(1+c) + c\, r_R(1+c) > -r_R \\
\leftrightarrow &d_R < \frac{c}{1+c} r_R,
\end{aligned}$$

where we have skipped some steps for brevity.

Claim 3: Finally, comparing R_K^* and R_r^* yields:

$$\begin{aligned}
&R_K^* > R_r^* \\
\leftrightarrow &\left(1 - \frac{d_R}{r_R}\right)(1-c)K_R > \left(1 - \frac{d_R}{(1-c)r_R}\right) K_R \\
\leftrightarrow &-(1-c^2)\frac{d_R}{r_R} - c(1-c) > -\frac{d_R}{r_R} \\
\leftrightarrow &d_R < \frac{1-c}{2-c} r_R,
\end{aligned}$$

where we again have skipped some steps for brevity. □

To illustrate these result we show the R-nullcline for a 30% cost in each parameter in Figure Figure A2. In Figure A2A we assume that $d_R = 0.3$ so that the cost in K_R has the largest impact. In contrast, in Figure A2B we set $d_R = 0.6$ so that the impact of a cost in K_R is now the smallest of the three, as predicted by Theorem A1.1. This illustrates further that the impact of a cost of resistance depends not just on the size of the cost and how it manifests itself, but also on the microenvironmental context.

How do the different possible manifestation of a cost affect the treatment dynamics? In Figure A2C, we show the AT treatment dynamics for a 10% cost in each of r_R, d_R and K_R for Tumour 1 ($(n_0, f_R) = (0.25, 0.999)$). Firstly, we consider the case of low turnover ($d_S = d_R = 0.3$). We find that only a cost in r_R is able to noticeably extend TTP. This is interesting, as it contradicts what one might predict based on Theorem A1.1: according to the theorem, the cost in K_R has the greatest impact on the population equilibrium, so that

one might expect the cost in K_R to yield the greatest improvement in TTP. However, as we can see from Figure A2C the resistant population takes longer to expand and more AT cycles can be completed (4 vs 5 cycles) when there is a cost in r_R. This is because the benefit of the reduced carrying capacity comes to bear only once the resistant population approaches its equilibrium. But at this point the tumour is already largely resistant so that there are few sensitive cells left which could constrain the expansion of the resistant cells. In contrast, the cost in r_R has an impact when the resistant population is still small and so can help to keep it smaller for longer. In the second case, we assume that turnover is high ($d_S = d_R = 0.6$) and in this case the order in which cost extends TTP coincides with what would be expected from Theorem A1.1. This is both because the costs in r_R and d_R reduce the population equilibrium further than the cost in K_R and because they significantly reduce the growth rate of the resistant population when it is small (Figure A2C). We conclude that the more a cost reduces the growth rate of the resistant population when it is small the greater will be the benefit for extending control under AT. So, a cost in r_R will bring the greatest benefits, followed by costs in d_R (provided the base turnover is sufficiently large) and K_R. This also agrees with the conclusions by [3] who found in their model that the benefit of AT increases the smaller the fitness of the resistant cells when they are rare.

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