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Spatial structure impacts adaptive therapy by shaping intra-tumoral competition

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Abstract: (1) Background: Adaptive therapy aims to tackle cancer drug resistance by leveraging intra-tumoural competition between drug-sensitive and resistant cells. Motivated by promising results in prostate cancer there is growing interest in extending this approach to other cancers. Here we present a theoretical study of intra-tumoural competition during adaptive therapy, to identify under which circumstances it will be superior to aggressive treatment, and how it can be improved through combination treatment; (2) Methods: We study a 2-D, on-lattice, agent-based tumour model. We examine the impact of different micro-environmental factors on the comparison between continuous drug administration and the adaptive schedule pioneered in the first-in-human clinical trial. (3) Results: We show that the degree of crowding, the initial resistance fraction, the presence of possible resistance costs, and the rate of tumour cell turnover are key determinants of the benefit of adaptive therapy. Subsequently, we investigate whether combination with treatments which amplify proliferation or which target cell turnover can prolong tumour control. While the former increases competition, we find that only the latter can robustly improve time to progression; (4) Conclusion: Our work helps to identify selection factors for adaptive therapy and provides stepping stones towards the rational design of multi-drug adaptive regimens.

1. Introduction

"Can insects become resistant to sprays?" - this is the question entomologist Axel Melander raised in an article of the same title in 1914 [1]. Melander had observed that at a site in Clarkson, WA, over 90% of San Jose scale, an insect pest affecting orchards, survived treatment with sulphur-lime, an usually highly effective insecticide [1]. If we were to ask the same question in cancer treatment we would be met with an equally resounding "yes": while for most cancers it is possible to achieve an initial, possibly significant, burden reduction, many patients recur with drug-resistant disease, or even progress while still under treatment. Drug resistance can develop in a number of ways, including genetic mutations which alter drug binding, changes in gene expression which activate alternative signalling pathways, or environmentally-mediated resistance [2–4]. In the clinic, the main strategy for managing cancer drug resistance is to switch treatment with the aim of finding an agent to which the tumour is still susceptible [3,4]. Similarly, Melander suggested it might be possible to tackle sulphur-lime resistance by switching to oil-based sprays [1]. But he also foresaw the possibility of, and the challenges arising from, multi-drug resistance [1]. As an alternative, Melander proposed that it might be possible to maintain insecticide sensitivity through less aggressive spraying as this would promote inter-breeding of sensitive and resistant populations and, thus, dilute the resistant genotype [1]. Allocation of drug-free "refuge" patches in the neighbourhood of plots in which an insecticide is used is one modality of modern pest management and is even required by law for the use of certain agents in the US (e.g. Bt-crops [5]).

Recently, the concept that treatment de-escalation can delay the emergence of resistance has found application also in oncology. Standard-of-care cancer treatment regimens aim to maximise cell

kill through application of the maximum tolerated dose, in order to achieve a cure. In contrast, an emerging approach called adaptive therapy proposes to focus not on burden reduction, but on burden control in settings, such as advanced, metastatic disease, in which cures are unlikely [6–9]. Eradication strategies free surviving cells from intra-tumoural resource competition which would otherwise inhibit resistance growth. Adaptive therapy aims to leverage this competition by maintaining drug-sensitive cells in order to avoid, or at least delay, the emergence of resistance [6,8]. A number of pre-clinical studies have demonstrated the feasibility of this approach in ovarian [7], breast [10], colorectal [11], and skin cancer [12]. Moreover, a clinical trial of adaptive therapy in metastatic castration resistant prostate cancer achieved not only a 10 month increase in median time to progression (TTP), but also a 53% reduction in cumulative drug usage [13]. Further clinical trials in castration sensitive prostate cancer, thyroid cancer, and melanoma are ongoing (clinicaltrials.gov identifiers NCT03511196, NCT03630120 and NCT03543969, respectively).

In addition to testing its feasibility, there has been significant interest in characterising the underpinning eco-evolutionary principles of adaptive therapy through mathematical modelling. We identify three key results. The first insight was derived from approaches which represent the tumour as a mixture of drug-sensitive and resistant cells modelled as a system of two or more ordinary differential equations (ODEs) with competition described by the Lotka-Volterra model from ecology [7,11,12,14–17] or by a matrix game [18]. These analyses have demonstrated that less aggressive treatment allows for longer tumour control under a range of assumptions on the tumour growth law (exponential: [11,14,16,18]; logistic: [7,12,14,16]; Gompertzian: [14–16]; dynamic carrying capacity: [11,17]), and the origin of resistance (pre-existing: [7,11,14–16,18]; acquired [12,15,16]; cancer stem-cell-based: [17]). Furthermore, this work predicts that adaptive therapy will be most effective if resistance is initially sufficiently frequent to make a cure unlikely and if the resistance fraction and resource supply (proximity to carrying capacity) are such that inter-specific competition with drug-sensitive cells is a dominant factor in restricting resistant population growth (see [16] for a comprehensive and formal summary of these results). The second key result is that while these conclusions broadly transfer to more complex, spatially-explicit tumour models, the spatial distribution of resistant cells is important [11,19]. Bacevic et al [11] showed in a 2-D, on-lattice, agent-based model (ABM) of a tumour spheroid that longer control is achieved if resistance arises in the centre of the tumour compared to when it arises on the edge. Gallaher et al [19] corroborated this result in a 2-D, off-lattice setting with resistance modelled as a continuum, and further demonstrated that tumour control was adversely affected by high cell motility and cell plasticity [19]. Thirdly, models focussed on metastatic prostate cancer have illustrated how these concepts may be realised in a specific disease pathology [13,17] and how we may enhance tumour control by using a multi-drug approach [17,20,21].

Using a minimalistic, sensitive/resistant Lotka-Volterra ODE model we recently examined the role of two factors not previously considered: resistance costs and cellular turnover [22]. The "cost of resistance" refers to the notion that drug-resistance may come at a fitness cost, such as a decreased proliferation rate, in a drug-free environment due to, for example, increased energy consumption. Such costs have, for example, been reported in colorectal cancer [11], breast cancer [19], and melanoma [12], and have been assumed to be necessary for the success of adaptive therapy [6,8]. We showed that this is not the case, and furthermore that the impact of a resistance cost depends on the rate of tumour cell death. Moreover, we found that higher rates of tumour cell death increased the benefit of adaptive therapy more generally as they amplified the effects of competition: the shorter a cell's life span and the fewer opportunities for division, the greater the impact if proliferation is inhibited by competition [22].

The aim of this paper is to examine whether these conclusions still hold true if space is taken into account. In addition, we aim to identify a "minimal spatial working model" for adaptive therapy, as both previous spatial models have made assumptions about nutrient supply [11] or cell cycle dynamics [19] which made these models more realistic, but also complicate identification of governing principles. In the following, we recast our previously derived ODE model into a 2-D, on-lattice model in which the

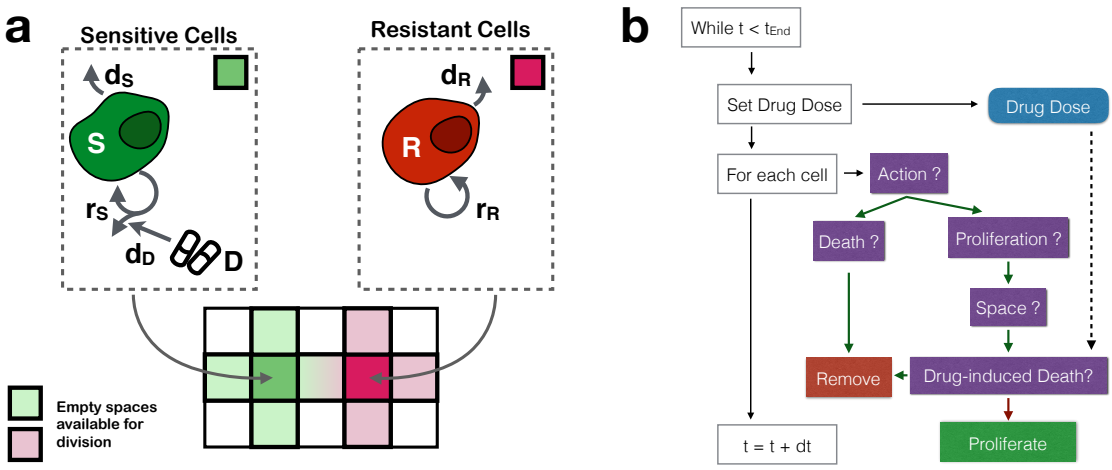

Figure 1. The agent-based tumour model: (**a**) The tumour is modelled as a mixture of drug-sensitive and resistant cells, where each cell occupies a square on a 2-D equi-spaced lattice. Cells divide and die at constant rates. Daughter cells are placed in empty squares in a cell's von Neumann neighbourhood. Drug will kill dividing sensitive cells at a rate d_D. (**b**) Flow diagram of the simulation algorithm.

tumour is assumed to be composed of drug-sensitive and resistant cells. We examine the roles of the initial resistance fraction, the proximity of the tumour to carrying capacity, resistance costs and cellular turnover and confirm the behaviour is consistent with our previous non-spatial results. Furthermore, we investigate the role of the initial cell distribution and find that clustering of resistant cells, consistent with a clonal model of resistance, facilitates tumour control whereas random distributions impede control. Finally, we ask how we may improve adaptive therapy by modulating proliferation or cell turnover through administration of a secondary drug. Overall, our work helps to clarify under which circumstances adaptive therapy may be beneficial, and contributes to the development of multi-drug adaptive regimens.

2. Materials and Methods

2.1. The mathematical model

Natural geno- and pheno-typical variation produces tumour cells which show a degree of drug-resistance even prior to drug exposure. This may manifest as an increased ability to persist and adapt to adverse conditions such as drug exposure, or, though perhaps more rarely, it may take the form of fully developed resistance [3,4]. Selective expansion and further adaptation of this population is thought to be the cause of treatment failure in patients.

We represent this process with a 2-D, on-lattice, ABM in which the tumour is composed of a mixture of drug-sensitive and resistant cells where, for simplicity, we assume that resistant cells are fully drug-resistant (Figure 1a). We choose an on-lattice, agent-based representation as it allows us to explore the role of space and cell-scale stochasticity in a tractable, yet generalisable, way. Each cell occupies a single site in an $l \times l$ square lattice, and behaves according to the following rules (Figure 1):

- Initially, there are a total of N_0 cancer cells spread randomly in the tissue, of which a fraction f_R is resistant.
- Sensitive and resistant cells attempt to divide at constant rates r_S and r_R (in units: day^{-1}), respectively. If there is at least one empty site in the cell's von Neumann neighbourhood, then the cell will divide and the daughter will be placed randomly in one of the empty spaces in the neighbourhood.
- Cells die at a constant rate d_T. For simplicity we will assume that both sensitive and resistant cells die at the same rate, $d_S = d_R = d_T$.

- Movement of cells is neglected.
- The domain is sufficiently small so that drug concentration $D(t) \in [0, D_{\text{Max}}]$ is assumed homogeneous throughout the tissue.
- A sensitive cell which is currently undergoing mitosis - that is, it has attempted division and has space available in its neighbourhood - is killed by drug with probability $d_D D(t)$, where $d_D < D_{\text{Max}}$.
- Dead cells are immediately removed from the domain.

We denote the number of cells in each population at time t by $S(t)$ and $R(t)$, and the total number by $N(t) = S(t) + R(t)$, respectively (Table 1).

We consider two treatment schedules:

1. Continuous therapy at the Maximum Tolerated Dose (MTD), D_{Max}: $D(t) = D_{Max} \forall\, t$.
2. Adaptive therapy as implemented in the Zhang et al [13] prostate cancer clinical trial: Treatment is withdrawn once a 50% decrease from the initial tumour size is achieved, and is reinstated if the original tumour size (N_0) is reached:

$$D(t) = \begin{cases} D_{\text{Max}}, & \text{until } N(t) < 50\% N_0 \\ 0, & \text{until } N(t) = N_0 \end{cases} \tag{1}$$

A flow-chart of our model is shown in Figure 1b, and further implementation details are given in Appendix A. We checked convergence (not shown), and performed a consistency analysis [23,24]. This showed that a sample size upward of 250 provides a representative sample size for our stochastic simulation algorithm (see Appendix B for details). The model is implemented in Java 1.8. using the Hybrid Automata Library [25]. Data analysis was carried out in Python 3.6, using Pandas 0.23.4, Matplotlib 2.2.3, and Seaborn 0.9.0. The code will be made available on-line upon publication.

2.2. Comparison with non-spatial model

To understand the impact of space we compare the agent-based model to the following ODE model which we have studied previously in [22]:

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

$$\frac{dR}{dt} = r_R \left(1 - \frac{R+S}{K}\right) R - d_T R, \tag{3}$$

$$N(t) = S(t) + R(t), \tag{4}$$

where K is the carrying capacity, and the initial conditions are given by $S(0) = S_0$, $R(0) = R_0$, and $N_0 = S_0 + R_0$, respectively. We set $K = l^2$ and used the same parameters as for the agent-based simulation otherwise. The equations were solved in the same fashion as in [22] using the RK5(4) explicit Runge-Kutta scheme [26] provided in Scipy.

2.3. Model parameters

We parameterise our model using values from the literature for prostate cancer (Table 1). We want to stress, however, that the aim of our work is to develop qualitative understanding, not to make quantitative predictions. As such, our predictions should be interpreted not in an absolute (treatment X will achieve a TTP of 8m), but in a relative fashion (treatment X will achieve a longer TTP than treatment Y because of mechanism Z).

Table 1. Summary of mathematical model variables and parameters.

Parameter	Description	Value	Comment
l	Grid size (Total number of sites: $l \times l$)	100	
dt	Simulation time step	1d	
$S(t)$	Number of sensitive cells	0.0 - l^2	
$R(t)$	Number of resistant cells	0.0 - l^2	
r_S	Sensitive cell proliferation rate	$0.027d^{-1}$	Adopted from [13].
c_R	Resistance cost (resistant growth rate: $r_R = (1 - c_R)r_S$)	0.0 - 0.5	Lower limit: [19]; Upper limit: assumption of no cost
d_T	Cell death rate (relative to r_S)	0.0 - 0.5	Lower limit: assumption of no turnover; Upper limit: see discussion in [22].
d_D	Drug-induced cell kill probability of sensitive cells at $D(t) = D_{\text{Max}}$	0.75	Adopted from [20].
$n_0 = \frac{N_0}{l^2}$	Relative initial cell density	0.25 - 0.75	Parameter sweep; values within this range reported by [27].
$f_R := \frac{R_0}{N_0}$	Initial resistant cell fraction	0.001 - 0.1	Parameter sweep; values within this range reported by [28].
$f_S := \frac{S_0}{N_0}$	Initial sensitive cell fraction	0.9 - 0.999	Determined by $1 - f_R$.

3. Results

3.1. Tumour control does not require resistance costs and is possible even if resistance arises in multiple locations

The aim of adaptive therapy is to delay disease progression by leveraging intra-tumour competition. While it has long been thought that fitness costs associated with resistance are pivotal for the success of this strategy, our prior non-spatial model shows adaptive therapy can succeed even in the absence of a cost [22]. To test whether this prediction holds true if spatial interactions between cells are taken into account, we compare matched simulations in which the same tumour (identical parameters and random number seed) is treated once with continuous treatment and once adaptively. Progression is determined according to the RECIST criteria as a 20% increase in tumour cell number relative to the start of treatment. Simulations for a representative example are shown in Figures 2a & b. Despite the absence of a resistance cost and despite a random initial distribution of resistant cells, adaptive therapy is able to prolong TTP by about 15.3m (95% CI: [14.81m,15.86m]). Examination of the cell distributions in the simulation over time clearly illustrates competitive release of the pre-existing resistant cells (Figure 2b).

3.2. High tumour cell density and low initial resistance fraction maximise competition from sensitive cells

While competition is thought to be a driving mechanism behind adaptive therapy, it is challenging to quantify its role in real tumours. This is because it is difficult to rule out confounding factors such as paracrine interactions or immune predation, and because no simple biomarkers exist for measuring the strength of competition. Within our mathematical model we have precise control over experimental conditions and we can measure quantities which are not accessible in real life. We seize this opportunity to examine how adaptive therapy modulates cell-cell competition for space. We measure what proportion of resistant cell divisions is blocked by the presence of another cell at different time points during treatment. During continuous treatment this fraction is initially low

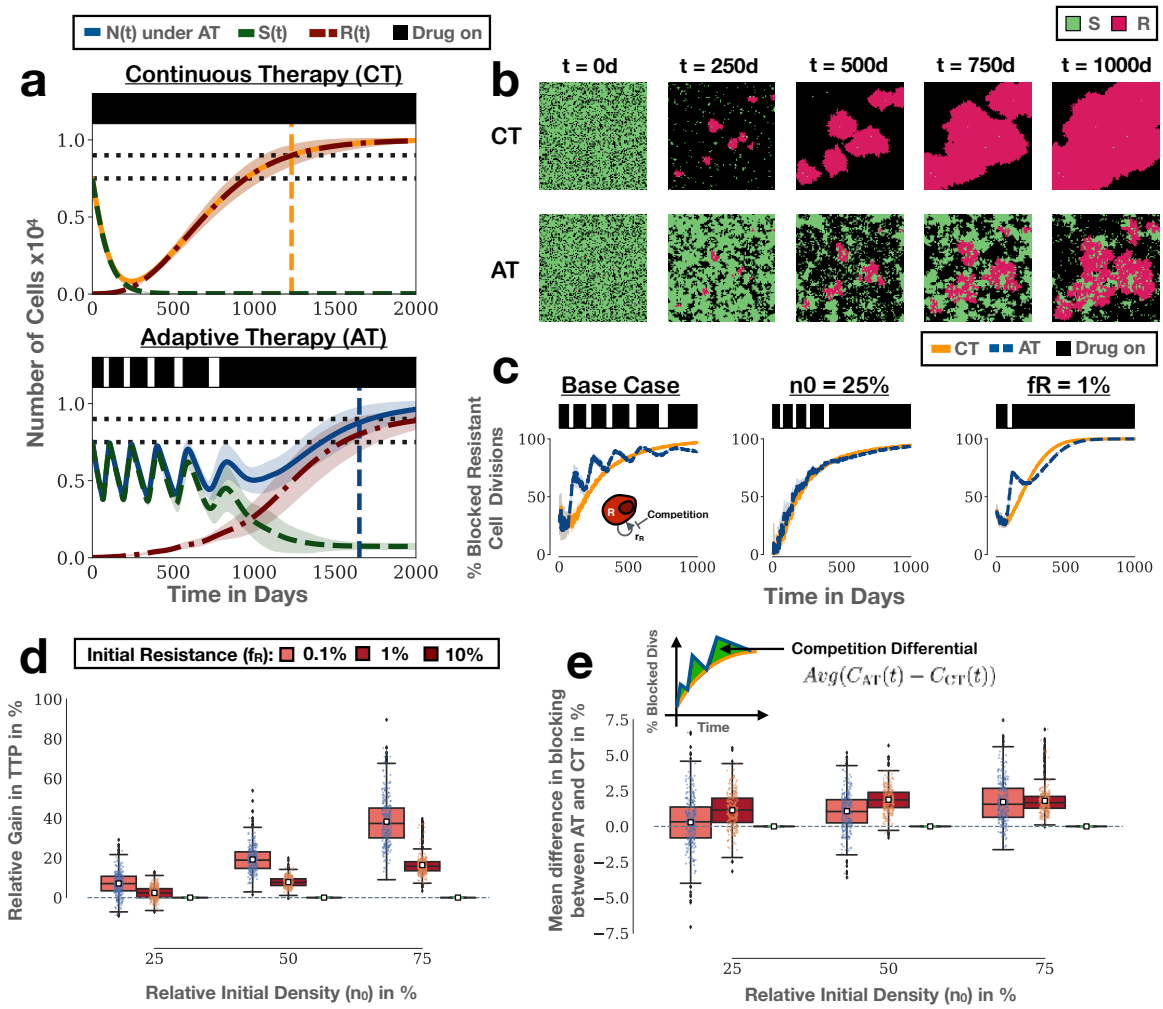

Figure 2. Adaptive therapy prolongs TTP by leveraging intra-tumoral competition and does not require a resistance cost: (**a**) Example of the treatment dynamics under continuous and adaptive therapy, showing a benefit for adaptive therapy despite an absence of resistance costs and despite random initial seeding of the cells ((n_0, f_R)=(75%,0.1%)). Shown are the mean and standard deviation (shading) of the tumour cell number (sensitive, resistant and total; 250 independent simulations). Black bars represents treatment. Horizontal dotted lines show the initial cell number, and the cell number at progression. Vertical lines and associated shading mark the mean, and the 1st and 3rd quartile of the distribution of TTP. (**b**) Snapshots from one of the replicates in (a) illustrating how adaptive therapy delays the competitive release of resistant tumour nests. (**c**) Adaptive therapy results in a higher proportion of blocked resistant cell divisions, explaining why resistance emerges more slowly in the simulations in (a). However, the ability to control depends on the initial tumour cell density (n_0; centre panel; (n_0, f_R)=(25%,0.1%)) and the initial resistance fraction (f_R; right panel; (n_0, f_R)=(75%,1%)). (**d**) Extension of TTP achieved by adaptive therapy relative to continuous therapy as a function of n_0 and f_R. Adaptive therapy yields the greatest benefit when tumours are close to their carrying capacity and resistance is rare. Negative values denote cases in which continuous therapy achieves a longer TTP (1000 independent replicates per condition). (**e**) High density and low resistance fractions maximise the difference in growth inhibition due to competition between continuous and adaptive protocols (1000 independent replicates per condition).

and gradually increases as the resistant tumour nests become larger, and cells at the centres of these nests are blocked by cells at the edge (Figure 2b & Figure 2c; left panel). While this gradual increase due to intra-specific competition also occurs during adaptive therapy, we additionally observe rapid

intensification of inhibition whenever treatment is withdrawn (Figure 2b & Figure 2c; left panel). This results in a level of growth inhibition which is consistently higher than that during continuous therapy until late in the course of treatment, and demonstrates how adaptive therapy leverages inter-specific competition.

Previous work by our group and others [15,16,19,22] has shown that the initial level of growth saturation of the tumour and the initial fraction of resistant cells are important determinants for the success of adaptive therapy. A parameter sweep over different values of n_0 and f_R confirms that a close proximity of the tumour to its carrying capacity and a small resistance fraction maximise the benefit of adaptive therapy also in the current model (Figure 2d). Moreover, consistent with this observation, we find that these conditions maximise the difference in the competition-mediated growth inhibition between continuous and adaptive therapy (Figure 2e). We conclude that high growth saturation due to close proximity to carrying capacity and small initial resistance fractions help to maximise the impact of inter-specific competition.

Moreover, we observe that n_0 and f_R have distinct effects on the competition experienced by resistant cells during adaptive treatment. When we reduce n_0, the tumour still cycles, but the impact of each cycle on the reduction of the resistant cell growth rate is greatly diminished (Figure 2c; middle panel). Conversely, if we increase the initial resistance fraction, treatment fails after only a single cycle. However, this single cycle still induces blocking of resistant cells and results in a benefit in TTP (Figure 2c; right panel). This suggests that such tumours might be able to be controlled for longer using a less aggressive treatment regimen. Indeed, treatment of the same tumour with an adaptive protocol in which drug is withdrawn at a 30% reduction in size, increases the time gained from 2.5m to 6.5m (Figure A1).

3.3. In the absence of turnover, a resistance cost does not increase the relative benefit of adaptive therapy

A key aim of this study was to investigate the role of resistance costs and cellular turnover on adaptive therapy. Figure 2a shows that neither costs nor turnover are necessary *per se* if the tumour is sufficiently close to its carrying capacity and resistance is rare. However, if these conditions are not met, then the benefit of adaptive therapy may be small, and in some cases longer control is, in fact, achieved with a continuous regimen (Figure 2d). This raises the question of whether in such cases a resistance cost may increase the benefit of adaptive therapy. We show simulations to test this hypothesis for two different initial resistance levels in Figure 3a. Addition of a resistance cost slows down the progression of the tumour under both therapies (Figure 3a), visible as a reduction in the size of resistant colonies (Figure 3b). An increased benefit of adaptive therapy is seen only for the case with the smaller value of f_R, and this increase is modest (Figure 3a; right panel). Mapping out the impact of resistance costs in more detail corroborates this result (Figure 3c). We conclude that in the absence of turnover, resistance costs may increase the absolute time gained but will have little or no impact on the relative benefit of adaptive therapy. Only those patients in whom AT also works without a cost will benefit.

3.4. High cell turnover increases the mean time gained by adaptive therapy by amplifying intra-tumoural competition

While cancer is known as a disease of increased cell proliferation, a large body of evidence suggests that cancers are also characterised by high rates of cell loss (e.g. [29–31]). We previously showed in a non-spatial model that high tumour cell turnover rates may increase the benefits of adaptive therapy [22]. To test whether this prediction holds true if spatial interactions are taken into account, we repeat our analysis from the previous section with a cell turnover rate of 30% relative to the proliferation rate, which is the value we previously estimated for prostate cancer [22]. For simplicity, we assume the same death rate for both sensitive and resistant cells and that dead cells are immediately removed from the domain. We find that the inclusion of cell death increases the average

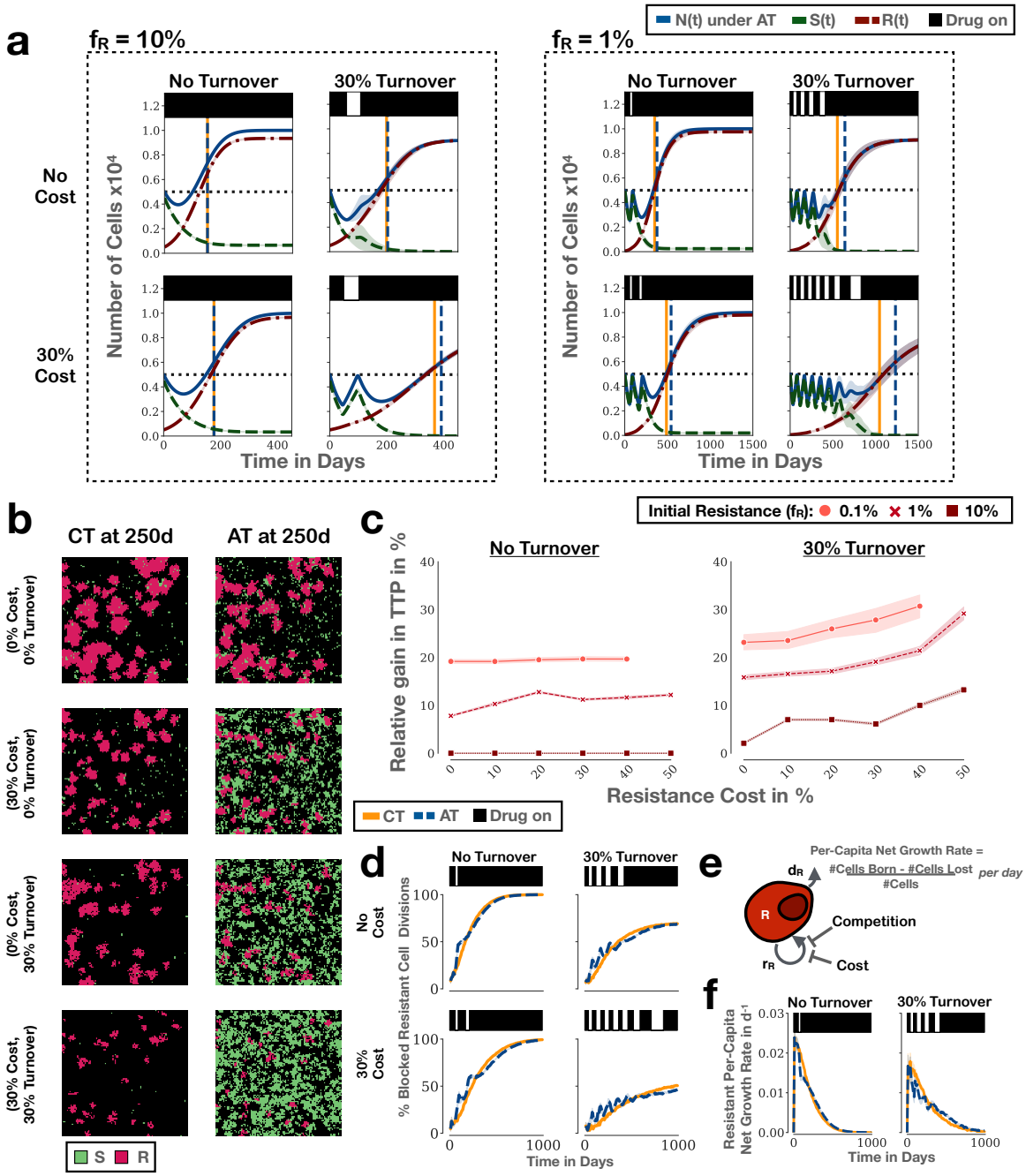

Figure 3. Resistance costs and high cellular turnover increase the benefit of AT: (**a**) Simulations illustrating the role of resistance costs and turnover on the treatment response to AT ($n_0 = 50\%$). Vertical lines and associated shading mark the mean, and the 1st and 3rd quartiles of the distribution of TTP. (**b**) Snapshots at time $t = 250d$ from one of the replicates in the right hand panel in a) ($(n_0, f_R) = (50\%, 1\%)$). All 8 simulations were started from the same initial conditions and treated either continuously or adaptively assuming the indicated combination of cost and turnover. (**c**) Relative benefit of adaptive therapy over continuous therapy as a function of resistance cost, resistance fraction, and turnover ($n_0 = 50\%$). This illustrates that turnover modulates adaptive therapy and increases the impact of a resistance cost. Shown are the mean (lines and points) and the 95% confidence intervals (shading). (**d**) Impact of resistance costs and turnover on blocking of resistant cell division for the simulations in (a) (right panel; $(n_0, f_R) = (50\%, 1\%)$). (**e**) The effect of competition on resistance controllability is determined by turnover. If turnover is high, then even moderate inhibition of cell proliferation may result in large reductions in per-capita growth rate. (**f**) Per-capita growth rate of resistant cells as functions of time, illustrating how turnover helps to amplify the effects of competition on the resistant population's net growth rate ($(n_0, f_R) = (50\%, 1\%)$).

number of adaptive therapy cycles, and with it the benefit of adaptive therapy (Figure 3a). In addition, turnover amplifies the effect of resistance costs (Figure 3a & c).

To explain why turnover facilitates the control of the drug resistant population, we first examine its impact on the tumour's spatial architecture. This shows that addition of turnover results, not only in smaller, but also in fewer emerging resistant nests, due to random extinction events (Figure 3b). The presence of a resistance cost further amplifies this effect (Figure 3b). Next we quantified the competition experienced by resistant cells in the presence and absence of turnover. We find that turnover reduces blocking of resistant cell divisions (Figure 3d). This illustrates that tumour control depends not only on the rate of proliferation of resistant cells but also on the rate of cell death, as it is the per-capita *net* growth rate of resistance which determines TTP (Figure 3e). While increased turnover frees up space for cell division (see gaps at the centre of nests in Figure 3b), it increases the impact of the blocking which does occur, as it limits the time, and so the opportunities, a resistant cell has for division. Indeed, comparing the per-capita growth rates of adaptive and continuous therapy we see that even though cell proliferation is less restricted with turnover, its impact on the per-capita proliferation rate is larger (Figure 3f). In summary, these results corroborate our hypothesis that the rate of tumour cell death is an important factor in adaptive therapy, and show how the impact of inhibition of cell proliferation depends on the rate of cell turnover.

3.5. Resistance emerging from single nests results in slower recurrence and higher inter-patient variability than resistance present in multiple sites

Having found that the spatial model qualitatively recapitulates our prior non-spatial results [22], we asked how space impacts tumour control with adaptive therapy. To do so, we compared simulations of the ODE and the agent-based version of our model for the same model parameters (Figures 4a & b). This shows that while the dynamics agrees qualitatively, there are quantitative differences in the predicted cycle lengths and TTP (Figure 4a). Specifically, if the initial fraction of resistance is high, progression occurs faster in the ABM, and conversely if the resistance fraction is small (Figure 4b). To further investigate this disparity, we examined the growth dynamics of the resistant population for three different initial sizes of the resistant population. Depending on the initial number of resistant cells, the population exhibits distinct growth kinetics (Figure 4c). When initiated from two cells, the resistant population expands from two nests and grows much more slowly than predicted by a logistic ODE model, as most cells are trapped by their neighbours (small surface to volume ratio). As the number of cells, and so the number of independent nests and the surface to volume ratio, is increased, the growth of the population speeds up until it exceeds that of logistic growth (Figure 4c). This explains why progression occurs quicker in the ABM when the initial number of resistant cells is high, and more slowly when it is small. This illustrates that while the logistic model is a useful first tool for modelling competition, it may be a poor descriptor of the growth kinetics in real tumours.

Next, we compared the relative benefit of adaptive therapy in the two models. We find that the ODE tends to predict a larger gain than the ABM, especially if turnover is included in the model (Figures 4a & d). Moreover, we observe variation in the possible outcomes in the ABM, with some patients even progressing faster on adaptive than on continuous therapy - an outcome not possible in the deterministic ODE model (Figure 4d and also Figure 2d). As we increase the domain size and so the total number of cells simulated, we find that this variation, and in particular the frequency of cases in which adaptive therapy performs worse than continuous therapy, are reduced (Figure 4e). This implies that when initial resistant cell numbers are small, stochastic events can cause large inter-patient variability which may cause difficulties in comparing adaptive and continuous therapy in these settings in practice. Overall, this shows that spatial interactions between cells may result in different growth kinetics to those expected from simple ODE models, with impacts on the predicted TTP and variability in outcome.

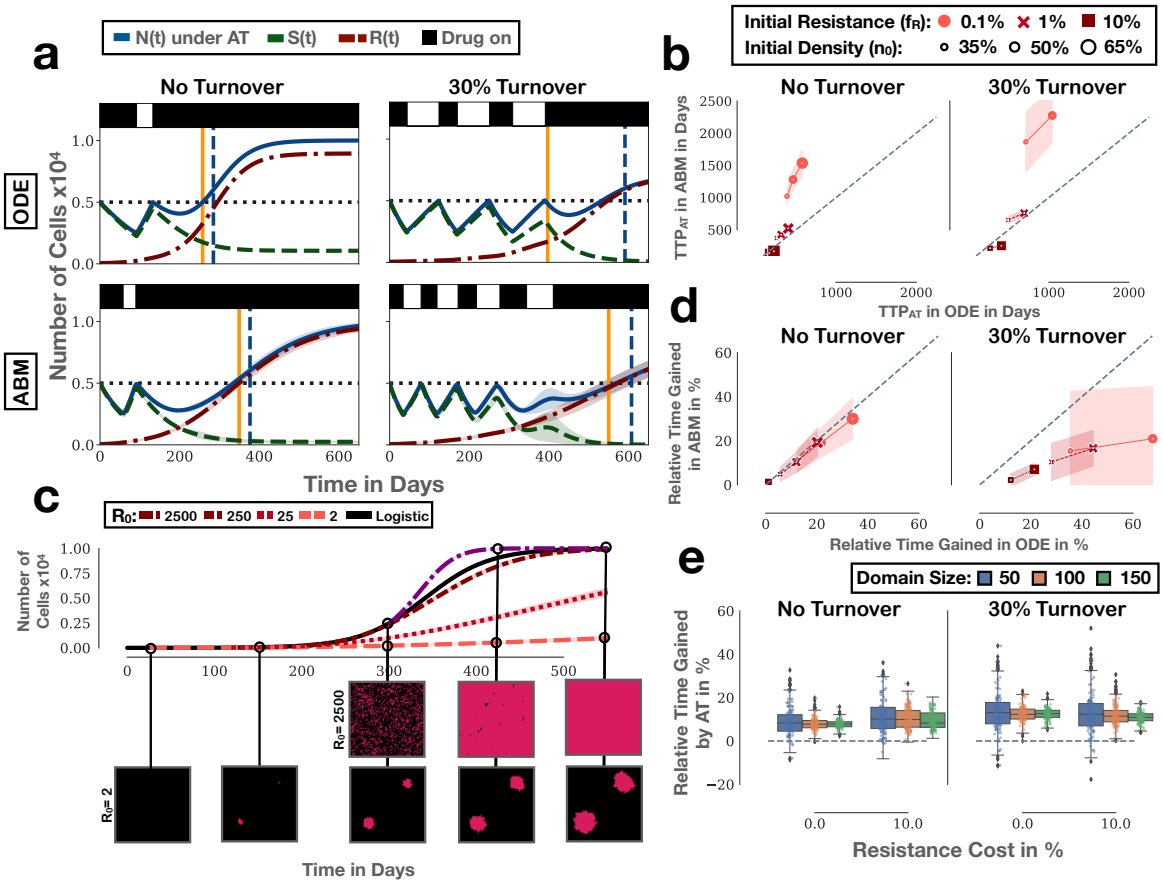

Figure 4. Comparing the spatial with the non-spatial model from [22]: (**a**) ODE and ABM simulations with the same parameters ($(n_0, f_R, c_R) = (50\%, 1\%, 0\%)$). For the ABM the mean and standard deviation of $n = 250$ independent replicates are shown. (**b**) Comparison of the TTP under adaptive therapy in the ODE and ABM ($c_R = 0$). The ABM predicts faster progression when initial resistant cell numbers are high, and slower progression when numbers are low ($n = 1000$ replicates of the ABM). In the presence of turnover, tumours starting from $n_0 = 65\%$ can not grow above the threshold defining progression and thus, no TTP can be obtained. (**c**) Depending on the initial cell number (R_0) the resistant population will grow sub- or super-logistically (logistic growth: black solid line). Resistant cells were seeded and grown in isolation and without drug ($n = 250$). To allow direct comparison with logistic growth, the agent-based curves were shifted to start at the time at which the logistic model reached the corresponding starting number of cells. (**d**) Comparison of the relative benefit of adaptive therapy ($(\mathrm{TTP_{AT}}\text{-}\mathrm{TTP_{CT}})/\mathrm{TTP_{CT}}$) in the two models. The ODE model tends to predict larger benefit than the ABM. Again, no TTP can be obtained for $n_0 = 65\%$. (**e**) Domain size (l) of the ABM reduces the variance in outcomes further corroborating the importance of the initial resistant cell number ($(n_0, f_R) = (50\%, 1\%)$; $n = 1000$ independent replicates).

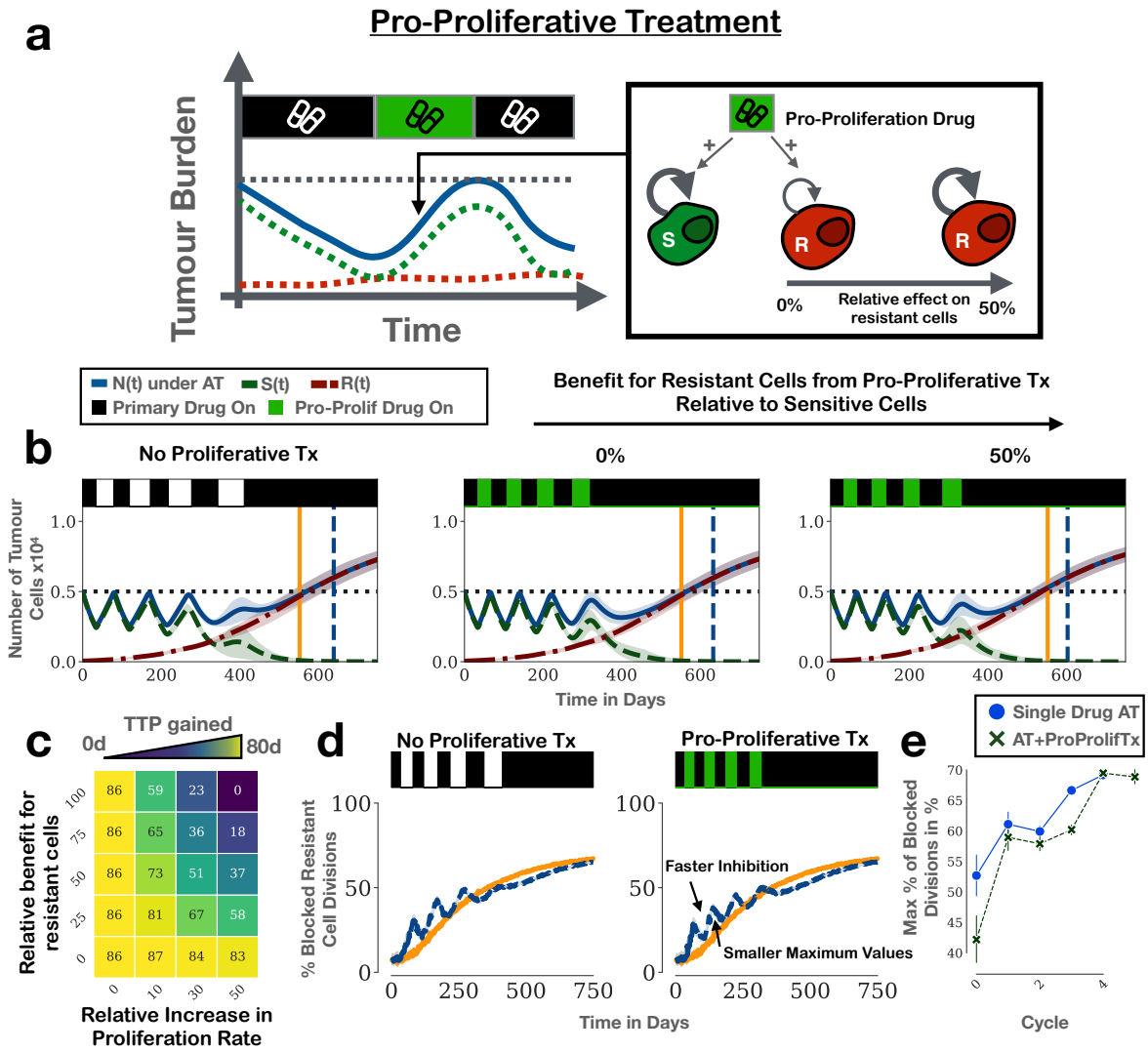

Figure 5. Increasing proliferation does not improve tumour control: (**a**) Sketch of the proposed pro-proliferation strategy which aims to increase competition by increasing the cell growth rate during the off-treatment phases of an AT cycle. (**b**) Simulations of AT treatment response without and with pro-proliferation treatment (30% increase in proliferation rate). Mean and standard deviation of $n = 250$ replicates, with vertical lines marking TTP (shading: 1st and 3rd quartile). Pro-proliferation treatment does not increase time gained by AT, and can even reduce it if resistant cells also benefit from the growth burst ($(n_0, f_R, c_R, d_T) = (50\%, 1\%, 0\%, 30\%)$). (**c**) Parameter sweep for different pro-proliferation treatment protocols for the tumour in (b), all confirming that pro-proliferation treatment fails to improve AT. (**d**) Comparison of the rate of resistant cell division blocking without and with pro-proliferation treatment for the simulations in (b) (0% benefit of pro-proliferation treatment for resistant cells). (**e**) The maximum fraction of resistant cell divisions blocked per AT cycle without and with pro-proliferation treatment in (d). Rather than increasing it, pro-proliferation treatment reduces competitive inhibition of resistant cells.

3.6. Optimising adaptive therapy: A cautionary tale for proliferation enhancing drugs

Having characterised the role of resistance costs and tumour turnover rates, we asked whether we could target these factors in order to develop more effective treatment protocols for tumour control. While it is not possible to engineer resistance costs, we hypothesised that it may be possible to intensify competition by promoting tumour re-growth during the off-treatment phase in order to more effectively encapsulate resistant cell nests. Thus, we developed a 2-drug "pro-proliferative" regimen

in which the primary drug is given adaptively as before, and in addition, a second drug is given, such as pro-angiogenic or hormonal treatment, which promotes cell proliferation during the off-treatment phase of the primary drug (Figure 5a). As it is plausible that pro-proliferative treatment might affect sensitive and resistant cells to different degrees (e.g. if a hormone-based stimulant is used from which only hormone-dependent cancer cells would benefit), we tested the scenario in which both cell types gain equally, as well as cases in which resistant cells benefit to a lesser degree.

In Figure 5b we show simulations of the proposed regime. Contrary to our expectations, we find that the pro-proliferation supplement does not improve tumour control. In fact, if we assume that resistant cells also benefit, then the tumour progresses more quickly than under the original single drug protocol. A parameter sweep confirms these observations for a wide range of treatment parameterisations (Figure 5c), and tumour characteristics (not shown). We conclude that within the scope of our model, care must be taken with stimulating proliferation as it may be detrimental to tumour control.

Why does the proposed regimen fail? To answer this question, we compared the competition-mediated growth inhibition of the single-drug and the pro-proliferative protocol (Figure 5d). As we hypothesised, pro-proliferative treatment does increase the rate at which resistant cells are blocked. However, because of the faster rate of re-growth, the primary drug is re-started sooner in the pro-proliferative protocol compared to the original protocol. As a result, there is not enough time to reach the same levels of growth obstruction seen in the single drug protocol (Figure 5e), so that the overall growth inhibition is reduced. This shows that promoting growth during the off-treatment phase does help to block the expansion of resistant nests. However, a more spatially targeted approach will be required in order to outweigh the negative impacts of faster tumour re-growth.

3.7. Targeting turnover to extend tumour control

Since leveraging proliferation did not yield the desired results, we next considered a multi-drug approach to target cell turnover. We hypothesised that by inducing additional cell death we could amplify the effect of competition-mediated growth inhibition on resistant cells, especially if a resistance cost was present. Similar to the pro-proliferation regimen, we considered a protocol in which the primary adaptive treatment is supplemented with a drug which increases cell death during the off-treatment phases (independent of division status; Figure 6a). An example of such a secondary drug may be a low dose chemotherapy. The response of drug-resistant cells to a drug to which they have not been previously exposed depends on the resistance and drug mechanism and can range from co-resistance to increased (collateral) sensitivity. For generality we will consider all three possibilities (increased, the same, or decreased sensitivity to the pro-turnover drug; Figure 6a). To implement this treatment we assume that during the primary drug-off phases sensitive and resistant cells have separate death rates $d_S = (1 + \gamma_T)d_T$ and $d_R = (1 + \rho\gamma_T)d_T$, respectively, where $\gamma_T \in [0, 1]$ describes the relative increase in turnover rate due to the secondary drug, and $\rho \in [0, 2]$ is the relative effect on resistant cells ($\rho < 1$: co-resistance; $\rho > 1$: collateral sensitivity).

In Figure 6b we show example simulations with the proposed regimen. As we increase the impact of the secondary drug on the resistant cells we observe an extension of tumour control (Figures 6b & c). Note though that even in the case of co-resistance there is a benefit for the combination protocol (Figure 6c). Next we examine the impact of this turnover-enhancing regimen on resistant cell growth. This shows that addition of the pro-turnover drug reduces the rate of blocking of resistant cells (Figure 6d). However, because of the higher turnover rate the impact of this blocking is amplified, so that the pro-turnover protocol achieves the lowest per-capita growth rates (Figure 6e).

So far we have compared our new combination regimen against single-drug continuous therapy. To control for the fact that we are now administering two drugs, we compare treatment in which the primary drug is given either continuously or adaptively, and both receive the same schedule of secondary drug (determined by the adaptive protocol). While the combination of continuous primary treatment and pro-turnover drug achieves better tumour control than continuous primary treatment

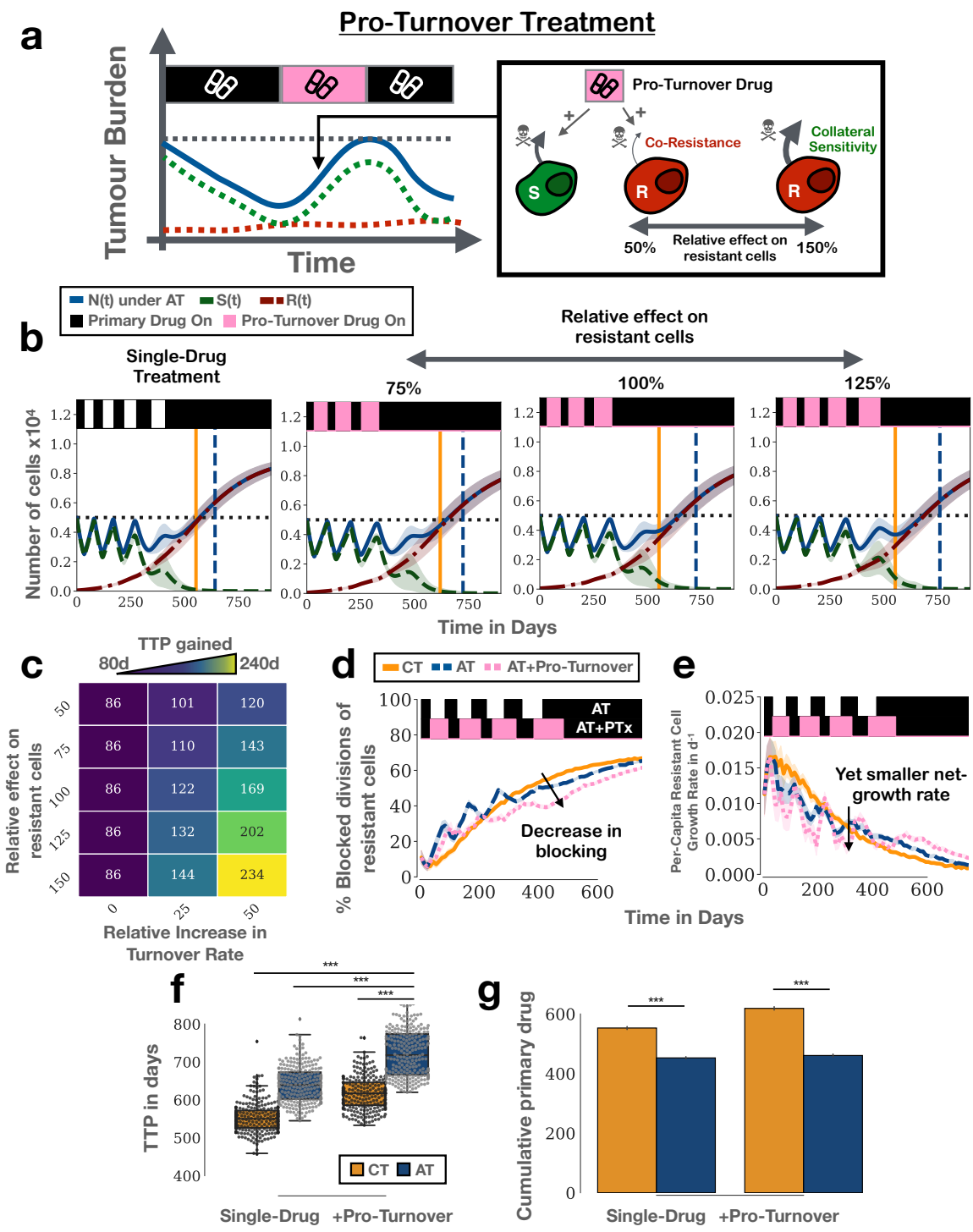

Figure 6. Increasing tumour control by increasing cell turnover: (**a**) Sketch of the proposed multi-drug regimen. In the off-phases of the primary drug a secondary drug, such as a low-dose chemotherapy, is given, which increases the turnover rate in the tumour. (**b**) Simulations of the pro-turnover protocol (50% increase in turnover). The stronger effect on the resistant cells, the longer the tumour can be controlled for ($(n_0, f_R, c_R, d_T) = (50\%, 1\%, 0\%, 30\%)$; $n = 250$ independent replicates of the ABM). (**c**) Parameter sweep of different pro-turnover protocols for the tumour in (b). The benefit is largest if resistant cells are affected more than sensitive cells, but even if this is not the case the pro-turnover regimen is still beneficial. (**d**) Effect of the new protocol on blocking of resistant cells (assuming equal effect of treatment on sensitive and resistant cells). (**e**) Effect of the same protocol on the per-capita growth rate of resistant cells. (**f**) TTP for single and combination treatments. *** indicate p-values in paired student t-tests of less than 1%. (**g**) Cumulative drug use of the primary drug for single and pro-turnover treatment. *** indicate p-values in paired student t-tests of less than 1%.

alone, the adaptive primary protocol still achieves the longest TTP (Figure 6f). Moreover, adaptive administration helps to significantly reduce the cumulative drug use in comparison to continuous therapy, especially if a secondary drug is given. This shows that also in combination treatment more drug is not necessarily better, and that more conservative but targeted drug application can achieve longer tumour control.

4. Discussion

Evolutionary theory has developed into a unified framework through which to understand cancer initiation, progression, and treatment response [32]. This has triggered the development of treatment strategies which aim to steer tumour evolution. Adaptive therapy is such an approach and seeks to control drug-resistance by exploiting intra-tumoural competition between treatment sensitive and resistant cells.

In this paper, we have studied adaptive therapy in a mathematical model of a small region of tumour tissue. We have aimed to keep our model as simple as possible in order to identify the key conditions required for adaptive therapy. As such we have represented the tumour region as a 2-D on-lattice, ABM in which tumour cells are classified as either drug-sensitive or resistant. We have shown that high initial tumour cell density, and low initial resistance fractions, maximise the benefit of adaptive therapy, assuming a cure is not possible. This prediction is consistent with previous non-spatial [11,15,16,18,22,33] and spatial theoretical studies [11,19], and experimental evidence in cancer [11] and bacteria [34].

Moreover, we corroborate our previous result [22] that a resistance cost is not required for adaptive therapy. We herewith demonstrate also in a spatial model that even in the absence of a cost we are able to slow the emergence of resistance by leveraging competition. Experiments have found that resistance costs depend on the genetic [12,35] and environmental context [22], so that it is likely that costs will vary between, and possibly also within, a patient. In combination with the work by Hansen et al [33] and Viossat and Noble [16], these results lend further support to the claim that adaptive therapy can work even if there is uncertainty about the presence of resistance costs in patients.

A question which was of particular interest to us was the role of cell death in adaptive therapy. We previously found in a non-spatial model that turnover may help resistance control by amplifying the impact of competition [22]. However, it was not clear whether this prediction would hold when space is taken into account, due to the way turnover will alleviate spatial obstruction. Our results corroborate the hypothesis that high cell turnover facilitates adaptive therapy, and we explicitly demonstrate its impact on competition. While tumour cell proliferation has been extensively studied, less is known about the rates of tissue turnover in tumours. Existing evidence suggests that cell loss rates are high in many cancers [29–31]. We propose further quantification of tumour cell death rates with a view towards developing a biomarker for selection of patients for adaptive therapy.

That being said, we also find that turnover increases the variability in treatment outcomes and increases the chance of adaptive therapy resulting in a worse outcome than continuous therapy. In addition, it has been suggested that tumours with higher turnover may be able to accumulate more resistance mutations than low turnover tumours [36]. This poses the question of how we may modify treatment in patients in whom single-drug adaptive therapy only yields little to no improvement in TTP. We investigate two strategies to prolong tumour control by increasing competition: Addition of 1) a pro-proliferative, or 2) a cytotoxic treatment in the off-phases of the primary drug. We find that addition of pro-proliferative treatment does intensify competition, but because it also shortens the off-periods it results in overall reduced competition-mediated growth inhibition. It is plausible that the discrete representation of space, absence of cell pushing and the lack of non-tumour tissue in our model negatively affect our ability to increase competition in this fashion, and these results should be re-examined in a more realistic tumour model. Nevertheless, this provides a cautionary example that the design of multi-drug adaptive therapies will require not only careful assessment of how drugs interact in the tumour, but also of how the action of one drug may change the schedule of the other.

As a second strategy we investigated an additional cytotoxic treatment which increases cell turnover. This succeeds in increasing TTP, which agrees with the work by West et al [20] and Brady-Nicholls et al [17] who have shown, via non-spatial modelling, that addition of chemotherapy can aid resistance control during androgen deprivation therapy in prostate cancer. We also note that patients in the initial prostate cancer adaptive therapy trial (NCT02415621) were receiving a continuous dose of Lupron in addition to adaptive Abiraterone which helps to starve cells of testosterone. Based on these results we advocate further research into multi-drug, adaptive regimens for the management of advanced, recurrent disease. Cure in these settings is often unlikely, and resistance to targeted agents evolves rapidly. Adaptive integration of targeted and chemotherapeutic therapies could help to reduce toxicity from cytotoxic treatment while also improving management of targeted therapy resistance.

Most theoretical work to-date has studied adaptive therapy using non-spatial models. This prompts the question of the extent to which results from these models translate to solid tumours which are often spatially heterogeneous. Review of published spatial and non-spatial models suggests good qualitative agreement, but a direct comparison has not been performed, to our knowledge. We have now analysed the same model in a spatial and a non-spatial formulation and can confirm good qualitative agreement in model behaviour. We observe, however, that the ODE model tends to predict greater benefit of adaptive therapy than the ABM. This is due to generally non-logistic growth dynamics in the ABM, where the resistant population emerges from different, independent nests (see Kimmel et al (in preparation) for further theoretical exploration of this phenomenon). Moreover, we show that the number of nests adversely affects TTP and the ability to control resistance with adaptive therapy. Due to the stochastic nature of our simulations we also observe variation in the benefit of adaptive therapy, as well as the possibility of failure of adaptive therapy ($TTP_{AT}<TTP_{CT}$). Variance increases with the average time gained, and depends on the grid size, which again highlights the importance of the number of independent nests driving progression. Challenges arising from such variation in treatment benefit in resistance management approaches have recently been discussed by Hansen et al [37]. Overall, these results imply that genetic resistance driven by rare pre-existing clones will be easier to manage than resistance emerging via phenotype switching or persister-cells, which is more likely to exist in multiple locations at once. Future research should investigate the implications of the emerging plastic view of resistance (e.g. [35]) for adaptive therapy.

In aiming to keep our model simple we have made a number of simplifying assumptions. We assume no movement and no pushing of cells, which has been shown by Gallaher et al [19] to reduce the benefit of AT, as it allows resistant cells to squeeze through surrounding sensitive cells. This may explain why Bacevic et al [11] were unable to control resistance in 2-D cell culture, even though our work here suggests that control should have been possible. Moreover, for computational reasons we restricted our analysis to a 2-D setting which is arguably more representative of *in vitro* cell culture than a 3-D human tumour. We hypothesise that the extra dimension will hinder tumour control as it will be more likely for resistant cells to find space to divide into. That being said, we have herein also neglected the role of non-tumour tissue which also acts as a competitor for space and resources in the tumour, and may help to control resistant subpopulations [18]. Future work should therefore study how adaptive therapy would play out in a more realistic 3-D setting, in order to identify challenges and opportunities arising from the impact on competition of a more complex spatial architecture, heterogeneous drug- and nutrient-distribution, and the non-tumour cell population.

It has been just over a century since Melander's first report of insecticide resistance in the San Jose scale. Today, the San Jose scale is approached through integrated pest management using a combination of carefully timed sprays, pheromone traps, and natural predators [38]. In a similar fashion, we advocate integration of different treatment modalities to *manage* drug-resistance in cancer. In this paper, we have consolidated and advanced our theoretical understanding of how competition between tumour cells may be leveraged by careful treatment modulation, and combination. Looking

ahead our insights should be tested in laboratory experiments in order to assess whether our theory can be applied in practice.

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Abbreviations

The following abbreviations are used in this manuscript:

ABM	Agent-based model
ODE	Ordinary differential equation
TTP	Time to progression

Appendix A. Stochastic simulation algorithm

A flow-chart of our model is shown in Figure 1b. We implement this model using the following fixed time-step stochastic simulation algorithm:

```
while t ≤ t_End do
    for all cells in the population do
        Select parameter set (k = sensitive or resistant)
        Let z_1 ~ U(0,1)
        if z_1 ≤ (r_k + d_k) dt then                          ▷ Check if an event occurs
            Let z_2 ~ U(0,1)
            if z_2 ≤ r_k/(r_k + d_k) then                     ▷ Check if cell divides
                if cell has space in neighbourhood then
                    Let z_3 ~ U(0,1)
                    Let σ = 1 if the cell is sensitive, otherwise σ = 0
                    if z_3 ≤ σ d_D D then                     ▷ Drug-induced death
                        Cell dies from drug
                    else                                      ▷ Cell division
                        Cell divides into randomly chosen spot
                        in neighbourhood
            else                                              ▷ Check if cell dies
                Cell dies
    t ← t + dt
```

Here, $\mathcal{U}(0,1)$ denotes the uniform distribution on $[0,1]$, and t_{End} the end time of the simulation in days.

Appendix B. Consistency analysis

A consistency analysis serves to determine the number of independent replicates required to obtain a sample of outcomes representative of the stochastic process. We adopt the protocol from [24] and apply it to corner cases of our parameter space, (n_0, f_R, c_R, d_T). The idea is to choose a sample size, n, obtain k independent samples of this size, and compare the k distribution of model outcomes. The aim is to find a value of n so that the k distributions are sufficiently similar and any one of them is representative of the others. We computed the difference in TTP for adaptive and continuous therapy for 10 samples of sample sizes $n = 10, 50, 100, 250, 500, 1000$, and 1500, respectively (68,200 simulations total). In Figure A2a we illustrate, for one parameter combination, how the thus obtained 10 outcome distributions for each value of n become almost indistinguishable for $n \geq 250$. To quantify consistency

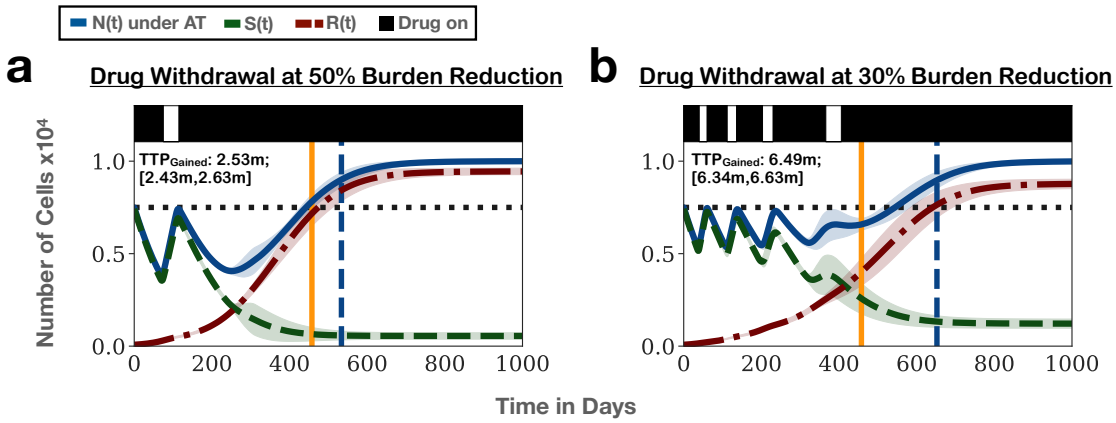

Figure A1. Comparison of two adaptive therapy protocols with different thresholds for treatment withdrawal. (**a**) Treatment withdrawal after a 50% tumour burden reduction. Lines and shading show the mean and standard deviation of the number of cells in each subpopulation and the total tumour size. Vertical lines denote the time of progression under continuous (yellow) and adaptive therapy (blue), respectively. Inset gives mean time gained and 95% confidence interval. (**b**) Treatment withdrawal after a 30% tumour burden reduction. This shows that less aggressive treatment, especially if resistance is prevalent prior to treatment, will result in longer tumour control, and matches the results obtained for non-spatial models [15,16,22]. Note though that we are not taking into account possible risks associated with the higher tumour burden under the 30% threshold algorithm. Parameters: $(n_0, f_R, c_R, d_T) = (75\%, 1\%, 0\%, 0\%)$; $n = 250$ independent replicates.

we measured the mean value of each distribution and the proportion of runs in which adaptive therapy performed worse than continuous therapy. This corroborates that $n \geq 250$ generates outcome distributions with very similar mean values and lower tails for a range of parameter combinations (Figure A2b & c). However, some parameter sets converge more slowly than others. Thus, we choose a value of $n = 1000$ for all analyses except for time-series plots (e.g. Figure 2a & b) for which we choose $n = 250$ for computational reasons.

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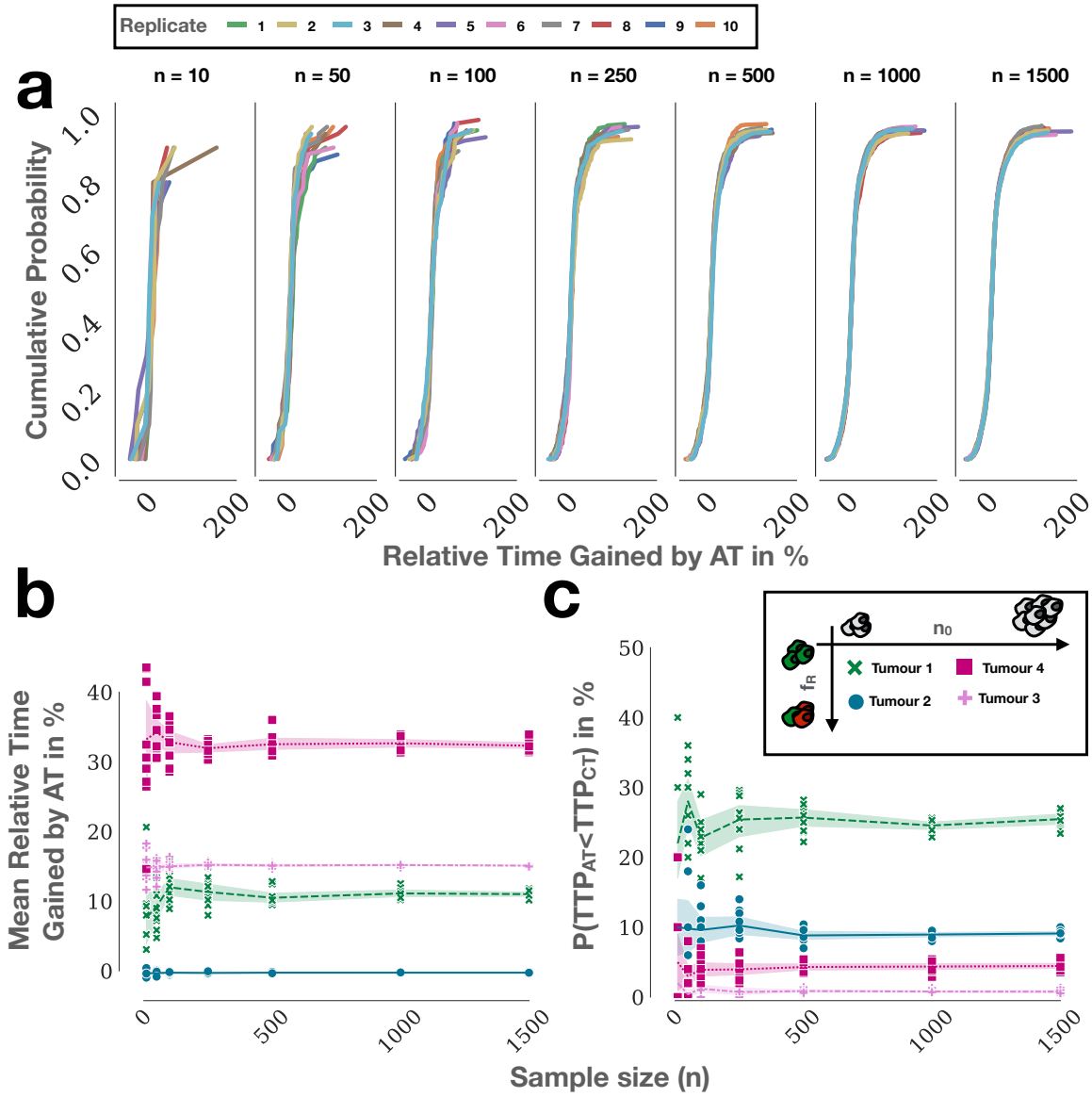

Figure A2. Representative results of the consistency analysis: (**a**) Increasing consistency in the distribution of time gained by adaptive therapy for one parameter set ($(n_0, f_R, c_R, d_T) = (25\%, 0.1\%, 0\%, 30\%)$ as the number of independent replicates per sample, n, is increased. For each value of n, 10 independent samples of sample size n were collected. (**b**) Mean value of the outcome distribution as a function of sample size, n, for four parameter sets (Tumour 1: $(n_0, f_R) = (25\%, 0.1\%)$; Tumour 2: $(n_0, f_R) = (25\%, 10\%)$; Tumour 3: $(n_0, f_R) = (75\%, 10\%)$; Tumour 4: $(n_0, f_R) = (75\%, 0.1\%)$;). Lines indicate the mean value, shading a 95% confidence interval. Markers show values of individual replicates. For sample sizes upwards of 250, the different replicates produce very consistent results. (**c**) Proportion of runs in which adaptive therapy failed as a function of the sample size, n for the same four parameter sets. Again for $n \geq 250$ we see consistent values between independent replicates indicating that a sample size $n \geq 250$ will yield a representative outcome distribution.

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