A theoretical study of the implications of resource competition for adaptive therapy of castration-resistant prostate cancer

A Thesis

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by

Harshavardhan BV

Indian Institute of Science Education and Research Pune
Dr. Homi Bhabha Road,
Pashan, Pune 411008, INDIA.

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Supervisor: Prof. Sutirth Dey

Certificate

This is to certify that this dissertation entitled A theoretical study of the implications of resource competition for adaptive therapy of castration-resistant prostate cancer towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Harshavardhan BV at Indian Institute of Science Education and Research under the supervision of Prof. Sutirth Dey, Professor, Department of Biology , during the academic year 2020-2021.

Prof. Sutirth Dey

Committee:

Prof. Sutirth Dey

Dr. M.S. Madhusudhan

This thesis is dedicated to this thesis

Declaration

I hereby declare that the matter embodied in the report entitled A theoretical study of the implications of resource competition for adaptive therapy of castration-resistant prostate cancer are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research, Pune, under the supervision of Prof. Sutirth Dey and the same has not been submitted elsewhere for any other degree.

Harshavardhan BV

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Abstract

The standard method for therapy against cancer is to administer cytotoxic drugs at the Maximum tolerated dose (MTD). This however, leads to competitive release of the drug-resistant cells and the tumour becomes unresponsive to further therapy. Adaptive therapy aims to maintain a steady population of drug-sensitive and prevent such competitive release by administering the drug at lower and fluctuating doses.

The success and failure of this method wholly lies the competition between the sensitive and resistant cells and the efficacy of the sensitive cells to suppress the resistant cells as a result. This competition is driven primarily by their requirement for limited resources in a tumour micro-environment. We have developed a mathematical model of the castration-resistant prostate cancer (CRPC) which emphasises on the dynamics of the resources, oxygen and testosterone, and their effect on the competitive dynamics of the cells. This is then further extended to the impact they would have on the outcomes of adaptive therapy.

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Chapter 1

Introduction

1.1 What is Cancer?

Cancer is a collection of diseases that is usually caused by an uncontrolled division of cells with the potential to spread to other parts of the body (NCI, 2021b). Cancer could be caused by extrinsic factors like tobacco usage, excess sun exposure or viral infections, as well as intrinsic factors like inflammation (Coussens & Werb, 2002; Trichopoulos et al., 1996). The underlying mechanisms of these causes usually involve genetic mutations or epigenetic changes that alter the DNA, which then trigger a cascade of events that eventually leads to uncontrolled growth of cells (GRØNBÆK et al., 2007; Moolgavkar & Knudson, 1981).

Cancer is among the highest non-infectious causes of death among human beings. In the year 2021, over 600,000 deaths are expected to be caused by cancer in the US alone (Siegel et al., 2021). Cancer systems have been of research interest for several decades due to the massive impact it has on human lives. Through such research, we have been able to understand the causes and mechanisms of how cancer arises, ways of prevention or early detection of cancer (Elmore et al., 2005; Goodman, 2015; Loeb et al., 1984), and develop new therapies and drugs that can target them more effectively and specifically while minimising unwanted collateral side-effects. While the mortality among some types of cancer have been reduced significantly, we have not been as lucky with other types of cancers and the overall mortality still remains unacceptably high.

1.2 Conventional therapy against cancer

The most common strategies to control cancer that form the frontline of current therapy include radiotherapy, immunotherapy, surgery, and chemotherapy (NCI, 2021a). Radiotherapy involves using ionising radiation to kill cancer cells. The high intensity radiation damages the DNA beyond repair, and this causes the cells to stop dividing and die. However, normal cells are also affected by the ionising radiation and the radiation therefore needs to be focussed to reduce collateral damage (Ahmad et al., 2012). Surgery involves removal of the tumour using highly invasive procedures. The tumour may be removed in its entirety if it is localised but partial removal may still be required to relieve patients of tumour burden when complete removal would be life threatening (Wyld et al., 2015). Immunotherapy involves activating the immune system of the body to target the cancer cells for killing. While components of the immune system on their own can detect and kill abnormal cells, cancer cells are able to evolve mechanisms to evade such immune suppression and emerging modes of immunotherapy are attempting to supplement the immune system to better target and fight against these cells (Couzin-Frankel, 2013). Chemotherapy involves administering cytotoxic drugs to kill cancer cells that frequently target all actively dividing cells, leading to the collateral loss of several stem cell populations (DeVita & Chu, 2008). Among more specific kinds of chemotherapy, there are different variants including hormone therapy which suppresses hormones required by cancer cells to survive, and targeted therapy which inhibits specific enzymes or antigens produced by cancer cells (Sawyers, 2004; Whitmore, 1956). Occasionally, therapeutic methods may also be devised which use multiple such drugs in combination. Depending on the type and stage of cancer, some of these strategies may not be effective.

Among chemotherapy, the standard clinical protocol, or the Standard of Care (SOC), followed for most cancers is to administer cytotoxic drugs at the maximum tolerated dosage (MTD) (Frei & Canellos, 1980). The aim of this method is to kill the maximum number of tumour cells as quickly or as early as possible. This minimises the tumour burden quickly and should lead to a better standard of living. Historically however, tumour relapse has been a problem as old as chemotherapy itself. From the earliest days of application of cytotoxic drugs to kill cancer cells, any remission achieved in the clinic has been temporary, and while the time taken for the cancer to grow back varies widely across tumour types, relapse occurs as new populations of cancer cells inevitably emerge (Baniel et al., 1995; Schilder et al., 1990). Even more problematically, these relapsed tumours have often turned out to

be resistant to the cytotoxic drug used earlier and the same drug can therefore no longer be used to control the new cancer population. Drug resistance in cancer, as with bacterial infections, is therefore an active area of research interest, with several approaches under development to predict and treat drug resistance emergence (Gao et al., 2017; Hall et al., 2009; Mokhtari et al., 2017).

Evolutionary reasoning has been applied extensively to explain and understand the emergence of drug resistance in bacterial populations (Davies & Davies, 2010). Like a bacterial population, a tumour would consist of cells with considerable heterogeneity in sensitivity to a cytotoxic drug. Under normal conditions, in the absence of therapy, these cells would compete with each other and keep the number of the resistant phenotype cells in check. Administration of this cytotoxic drug, particularly at a high dose with the goal of maximising cell kill would first kill off the most sensitive cells, which leads to a "competitive release" of the resistant phenotype (Scott & Marusyk, 2017). The resistant phenotype now grows into the space freed by the killing of sensitive cells without inhibition and takes over the entire population local niche. These resistant phenotypes don't respond to further dose administered and the therapy fails. This is illustrated in Figure 1.1. It is worth noting that competitive release could happen for other methods of therapy as well, if there are resistant phenotypes for that particular therapy method present in the population.

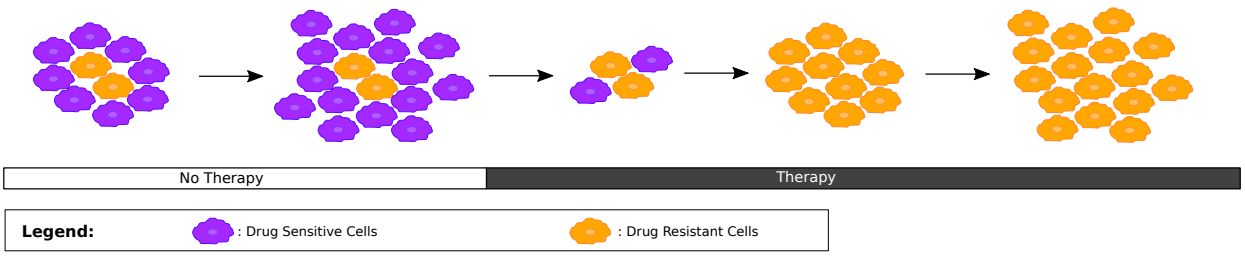


Figure 1.1: Illustration of competitive release under SOC

1.3 Adaptive therapy

When competitive release happens, one could try to combat the cells with another drug or therapy method. However, these cells could potentially be resistant to the new drug as well and developing new drugs is research intensive. It would therefore be preferable to avoid such competitive release in the first place.

Adaptive therapy (AT) is one such novel approach to therapy under development to avoid competitive release. In AT, the cytotoxic drug is administered at lower and fluctuating doses in which the drug is administered at some lower dose for some duration, which would be followed by a drug holiday (Gatenby et al., 2009). This doesn't kill off all the sensitive cells, and allows some fraction of them to remain in the micro-environment to prevent the resistant cells from taking over; this function of the sensitive cell fraction is thought to be due to the pressure of ecological competition they impose on resistant cells by competing with them for space and resources like oxygen. This competitive inhibition of resistant cells by the sensitive ones occurs during the drug holiday, when sensitive cells are not growth-limited by the drug. The tumour burden could then be kept under control as subsequent drug doses counteract the regrowth of sensitive cells during the drug holiday, as illustrated in Figure 1.2. The dosing schedule is therefore a central aspect of AT and must be designed to preserve the competitive inhibition of drug-resistant cells. One of the many regimes envisioned so far in the field uses tumour size as a metric to decide the timing of the dose and when the drug is withdrawn. The challenge with designing AT regimens is to balance the inhibition of resistant phenotype against absolute reduction of the overall tumour size. Adaptive therapy can be influenced by a wide range of factors that affect the dynamics and functioning of the system, and many of these parameters are under active investigation, including but not limited to cell turnover, phenotypic heterogeneity, spatial constraints and fitness cost for resistance (Bacevic et al., 2017; Gallaher et al., 2018; Strobl et al., 2020; Viossat & Noble, 2020).

The promise of tumour control notwithstanding, it is worth noting that AT may not be able to achieve control indefinitely. It only attempts to improve the survival time and/or the time to relapse compared to other regimens and largely ignores the possibility of a cure, sometimes even where the standard of care method might yield better results. The patient has to live with the tumour for the rest of their life and other complications could arise due to this. Models are therefore being developed that could contribute to this decision-making process before therapy. The decision-making model would choose between the aggressive standard-of-care or adaptive therapy to either attempt cure or contain respectively after assessing the probability of cure as well as the risks and complications of maintaining a high tumour burden based on the composition of tumour (Hansen & Read, 2020).

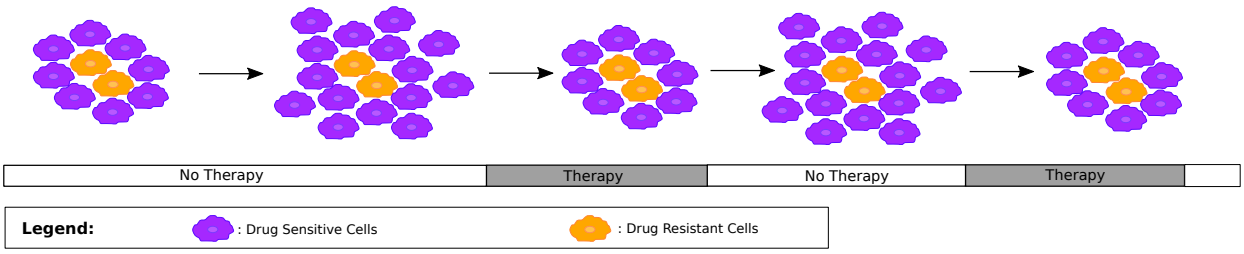


Figure 1.2: Illustration of control under AT

1.4 Importance of competition in adaptive therapy

The only way of controlling the resistant phenotype for a fixed drug under AT is through competition by the sensitive cells. Therefore, the success of AT in containing the tumour depends on the effectiveness of competition between sensitive and resistant cells. It is frequently assumed that resistant cells are required to have an inherent growth disadvantage in the absence of the drug (cost of resistance) for AT to be successful, but it has been shown that the survival time can be prolonged by competition between the cells even without a cost of resistance (Strobl et al., 2020). Given that competition for resources between cell types can play such a central role in the success of AT regimens, a more detailed examination of how such competitive dynamics could play out would be a valuable addition to the existing literature. More specifically, a description of the competitive interactions between cancer cell types that is framed in terms of explicit resource dynamics along with cell population dynamics is a significant gap worth addressing.

1.5 System of Study

The castration resistant prostate cancer (CRPC) was chosen to be the system of study. The CRPC system already has a history of AT work done on it, although in different contexts (Cunningham et al., 2018; Zhang et al., 2017).

Prostate cells express androgen receptors (ARs) that require testosterone or its metabolite, 5-dihydrotestosterone to activate. Activated ARs bind to promoters of genes responsible for proliferation (Heinlein & Chang, 2004). Without testosterone, proliferation is halted and the cells die of apoptosis. When cancerous cells evolve from prostate cells, the AR mecha-

nism is preserved and the cancer remains testosterone-dependent. However, mutant clones in prostate cancer can acquire mutations that allow them to synthesise their own testosterone independent of systemic supply, and these mutant clones are therefore the basis for acquisition of resistance to all forms of castration. Other mutations have also been shown in cancer cells to become entirely testosterone-independent, by acquiring mutations in the AR that make them constitutively active independent of testosterone.

This system is therefore usually modelled as consisting of three different types of cells: T^+, T^p and T^-. T^+ is the baseline population for prostate cancer which requires testosterone for survival and is dependent on systemic supply of the hormone, with no internal sources. The standard therapy for prostate cancer is castration or androgen deprivation therapy (ADT) which blocks external production of testosterone and would kill the T^+ cells in a normal castration-sensitive prostate cancer. Castration-resistant prostate cancer cells are modelled as T^p cells that can produce testosterone and sustain the T^+ cells. T^p cells are also dependent on testosterone, and they synthesise testosterone from cholesterol through upregulation of the enzyme, CYP17α (Dillard et al., 2008). Finally, the hormone-independent non-producing fraction of the tumour is modelled as the T^- cells, which do not require testosterone for cell growth. Abiraterone is a drug developed to specifically target castration resistance in prostate cancer that inhibits the CYP17α enzyme and can be effective against both T^+ and T^p, but would have no effect on T^-, as expected based on the functional differences between the three cell types.

1.6 Goals of the Project

This project has the following objectives in the context of adaptive therapy in the castration-resistant prostate cancer system.

1. A model must be built of castration-resistant prostate cancer that includes explicit formal descriptions of resource dynamics alongside the dynamics of the three cell types detailed above. The rationale for this is to allow competition in the model to operate through the concentrations of the various resources that constitute the environment. This eliminates the need to estimate values of Lotka-Volterra type competition coefficients that are otherwise difficult to define and interpret based on clinical data.

2. The model has three cell types which can be studied in stages, starting with two pairs: $T^p - T^-$ and $T^p - T^+$. The third pair, $T^+ - T^-$ is a trivial case as T^+, with no internal or external sources of testosterone, would go extinct with or without T^-. The reason for starting with the pairwise interactions is to come up with a set of simpler observations about how resource concentrations affect competition between two cell types. These simpler observations can then be used to organise and interpret the outcomes of the full three-way competition between T^p, T^+ and T^-. Such a stepwise approach gives us a way to reduce the complexity of a system with three cell types and multiple resources, with each resource produced and consumed differently across the cell types.

   (a) While the differences of production and consumption of each resource between each cell type is the qualitative basis for interactions between them, these differences do not necessarily constitute competitive strategies, as the status of each cell type with respect to how it interacts with each resource is fixed. For example, if T^p is dependent on testosterone for growth, the magnitude of its sensitivity to a low concentration of testosterone in the environment is the basis of its competitive strategy. While T^p cannot become fully independent of testosterone, the minimum concentration of the hormone required for T^p can be moved around to produce different strengths of competition for the hormone with T^+. Strategies of competition are therefore based on the extent to which a given cell type is limited by a resource, given that its mode of dependence is already defined.

   (b) Given this definition of competitive strategies, the model can be used to explore competition in two separate contexts, depending on whether all three cell types have the same competitive strategy or each cell type is adopting a strategy unique to that type. Specifically, this is encoded in terms of the level of limitation of a given resource for each cell type. Additionally, both the initial population size and the relative proportion of each cell type in the initial tumour population would have significant effects on the outcomes of competition. Each of these strategies would therefore be studied across a range of population sizes and seeding ratios.

   (c) Based on the previous two points, a comprehensive picture of competitive dynamics in the system can be assembled, with an understanding of how this picture is constructed based on the availability and utilisation of various resources.

3. The application of therapy affects the ecological balance of this system in very specific ways, and the understanding of competitive interactions in the system based on

resource dynamics also allows for outcomes of therapy to be explained in terms of how it affects competition.

(a) Standard-of-care (SOC) treatment is the baseline approach against which adaptive therapy (AT) is to be evaluated. SOC involves constant application of the drug at the maximum tolerated dose (MTD). AT design includes at least two parameters- the threshold population sizes at which therapy must be turned on and off, and the gap between these two thresholds. Together, they form the therapy window within which the tumour must be contained. This window can be placed at a large or small population size, and can be wide or narrow. This would form the basis for the standardisation of the AT regimen used for the rest of the model.

(b) Once a standard AT regime is found, it would be applied to the model with all three cell types. As with the study of competition, the standardised AT regimen will be applied to all strategy conditions, across a range of population sizes and seeding densities.

Chapter 2

Methods

2.1 System of Equations

The system of study was modelled using coupled Ordinary Differential Equations (ODEs). The model is based on a logistic framework modified with a dynamic carrying capacity that depends on the environmental conditions. The "environment" consists of the resources, oxygen and testosterone which have their own equations for production and consumption. We make the simplifying assumption that every other resource required by cells are present in non-limiting concentrations. Additionally, the cell types were assumed to not mutate and hence cannot change their types. No spatial structure is considered and the system is assumed to be well mixed and the resource available in bulk for all the cells.

The ODEs for population size of a cell type is given in Equation 2.1. The equation is such that the population increases by a maximum growth rate $r_{i,max}$ and reduces by a maximum death rate δ_i. The effective growth rate decreases as the total population approaches a maximum limit while the effective death rate stays the same. This maximum limit for the total population varies between 1 to $K_{i,max}$ and varies depending on the resource availability as a function of the form as given in Equation 2.2 and visualised in Figure 2.1.
For $i \in \{T^+, T^p, T^-\}$

$$\frac{dy_i}{dt} = r_{i,max}(dtx)y_i(1 - \frac{\sum_j y_j}{1 + K_{i,max}f_i(O_2)f_i(test)}) - \delta_i y_i \tag{2.1}$$

The functional dependence on resource $f_i(res) \in [0, 1]$. Below the lower limit, $ll_{res,i}$ the function is 0, representative of no growth, and increases linearly above it upto the upper limit, $ul_{res,i}$ and the function saturates to 1, representative of the maximum growth, for any resource levels above that.
For $res \in \{O_2, test\}$

$$f_i(res) = \begin{cases} 1 & \text{if } ul_{res,i} \leq res \\ \frac{res - ll_{res,i}}{ul_{res,i} - ll_{res,i}} & \text{if } ll_{res,i} < res < ul_{res,i} \\ 0 & \text{if } res \leq ll_{res,i} \end{cases} \tag{2.2}$$

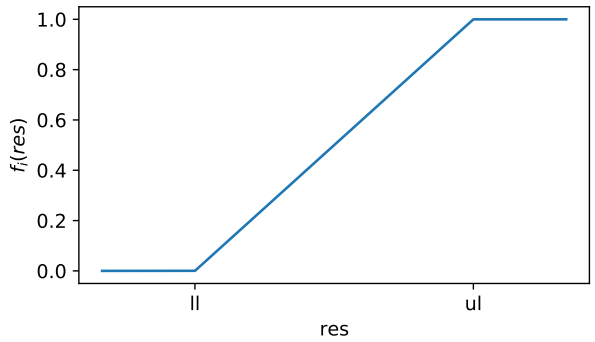


Figure 2.1: $f_i(res)$

The ODE for oxygen is given in Equation 2.3. This involves a term for external production that increase oxygen levels constantly at a rate p_{O_2}, a term for uptake by all cells where they decrease oxygen levels at a rate $\mu_{O_2,i}$ and a term for decay where oxygen level decreases at a rate λ_{O_2}.

$$\frac{dO_2}{dt} = p_{O_2} - \sum_i \mu_{O_2,i} y_i - \lambda_{O_2} O_2 \tag{2.3}$$

The ODE for testosterone is given in Equation 2.4. The form is similar to that of oxygen, with the difference being production being done by T^p cells at a rate p_{test} here.

$$\frac{dtest}{dt} = p_{test}(abi) y_{T^p} - \sum_i \mu_{test,i} y_i - \lambda_{test} test \tag{2.4}$$

Note that these equations are defined only for positive values of cell count and resource level to be biologically relevant. To mitigate the problem of having a continuous variable for cell count, $y_i < 1$ is defined as extinction of the cell type i and $y_i = \frac{dy_i}{dt} = 0$ in such a case.

2.2 Therapy

For implementation of therapy, production rate of testosterone and growth rate of the cells are governed by the dose of abiraterone abi and docetaxel dtx respectively as given in Equation 2.5 and Equation 2.6. Therapy is modelled as a boolean value, where 1 represents dose at MTD and 0 represents no dose.

$$p_{test}(abi) = \begin{cases} p_{test,max} & \text{if } abi = 0 \\ p_{test,min} & \text{if } abi = 1 \end{cases} \tag{2.5}$$

$$r_i(dtx) = \begin{cases} r_{i,max} & \text{if } dtx = 0 \\ r_{i,min} & \text{if } dtx = 1 \end{cases} \tag{2.6}$$

The dosing scheme for standard-of-care is given in Equation 2.7. Here, the dose is applied at MTD at all times from the start of the simulation regardless of the population size.
For $dose \in \{abi, dtx\}$

$$dose(x,t) = 1 \quad \forall\, t, x \tag{2.7}$$

The dosing scheme for adaptive therapy is given in Equation 2.8. A binary mode of adaptive therapy is considered here, where dose is applied at MTD when the population size exceeds the on threshold and stays on until the population size falls below the off threshold, after which it is turned off.

$$dose(x,t) = \begin{cases} 0 & \text{if } dose(x, t-\Delta t) = 0 \text{ and } x < on \\ 1 & \text{if } dose(x, t-\Delta t) = 0 \text{ and } x \geq on \\ 1 & \text{if } dose(x, t-\Delta t) = 1 \text{ and } x > off \\ 0 & \text{if } dose(x, t-\Delta t) = 1 \text{ and } x \leq off \end{cases} \tag{2.8}$$

2.3 Constraint equations and parameters from literature

Table 2.1 gives a brief description of the parameters from the above equations, the values used, and the sources for these values where applicable. Note that all the resource parameters are normalised to "Tissue levels of that resource" as obtained from the literature sources cited. The cell lines of LNCaP, 22Rv1 and PC3 were considered to correspond to the T^+, T^p and T^- cells respectively when obtaining literature values.

Constraint equations given below were used to determine the values of some parameters for which direct sources were not available.
Equation 2.9 is obtained from solving Equation 2.1 from N_0 to $2N_0$ under the assumption that resources are not limiting and y_i is small. This constraint along with doubling time and death rates obtained from literature can be used to get the growth rate.

$$r_{i,max} = \frac{ln(2)}{\tau_{d,i}} + \delta_i \tag{2.9}$$

Equation 2.10 is obtained from setting Equation 2.1 = 0 under the assumption that equilibrium is reached with only one cell type present and resources are not limited. This constraint along with an assumed equilibrium value of 10000 for the cells, growth and death rate obtained from above can be used to get the maximum carrying capacity for that cell type.

$$K_{i,max} = \frac{r_{i,max}}{r_{i,max} - \delta_i} y_i^* \tag{2.10}$$

Equation 2.11 is obtained from setting Equation 2.3 = 0 under the assumption that equilibrium is reached with only T^- cell type present. This constraint along with an assumed equilibrium value of 1 for oxygen and 10000 for the cells, and uptake and decay rates from literature can be used to get the production rate of oxygen.

$$p_{O_2} = \lambda_{O_2} O_2^* + y_i^* \mu_i \tag{2.11}$$

Equation 2.12 is obtained from setting Equation 2.4 = 0 under the assumption that equilibrium is reached with only T^p cell type present. This constraint along with an assumed equilibrium value of 1 for oxygen and 10000 for the cells, decay rates from literature can be

used to get the production rate of testosterone.

$$p_{test,max} - \mu_{test,T^p} = \frac{test^* \lambda_{test}}{y^*_{T^p}} = 4 \times 10^{-4} \tag{2.12}$$

Equation 2.13 is the same as Equation 2.12 with a lower equilibrium value of testosterone with abiraterone therapy.

$$p_{test,min} = \frac{test^*_{abi} \lambda_{test}}{y^*_{T^p}} + \mu_{test,T^p} \tag{2.13}$$

Equation 2.14 is the rearranged version of Equation 2.10 with a lower equilibrium value for the cells with docetaxel therapy.

$$r_{i,min} = \frac{K_{i,max}}{K_{i,max} - y^*_{i,dtx}} \delta_i \tag{2.14}$$

2.4 Code Implementation

The code is written in Python 3 and with dependencies of numpy, scipy, pandas, matplotlib and seaborn libraries. The system of equations were solved numerically by the LSODA algorithm provided by the `scipy.integrate.ode` function. The code is designed to iterate over the different parameters of a set parallely over multiple threads, however, the actual solver is sequential and single threaded.

The code, at each time step checks if the values are non-negative and sets them to 0 if it is the case. This is since the equations are not defined in these range of values and numerical errors can give rise to negative values. A similar implementation is done for $y_i < 1$.

The source code along with the data is available at the following Github repository: https://www.github.com/harshavardhan-bv/cancer-compe-strat.

Figure 2.2: QR code for the Github repository

Parameter	Description	Value(s)			Source(s)
y_i	No. of cells of cell type i	N/A			N/A
$r_{i,max}$	Population growth rate of cell type i	T^+	2.84×10^{-3}	min^{-1}	Equation 2.9
		T^p	2.79×10^{-3}	min^{-1}	
		T^-	6.23×10^{-4}	min^{-1}	
$r_{i,min}$	Population growth rate of cell type i under dtx therapy	T^+	2.55×10^{-3}	min^{-1}	Equation 2.14
		T^p	2.54×10^{-3}	min^{-1}	
		T^-	2.06×10^{-4}	min^{-1}	
δ_i	Population death rate of cell type i	T^+	2.5×10^{-3}	min^{-1}	(Jain et al., 2011)
		T^p	2.5×10^{-3}	min^{-1}	
		T^-	1.6×10^{-4}	min^{-1}	
$K_{i,max}$	Maximum Carrying capacity, coming up through the environment/resources	T^+	8.35×10^4		Equation 2.10
		T^p	9.62×10^4		
		T^-	1.34×10^4		
$f_{i,res}$	Functional dependence of cell type i on resource res, normalised to 1	$f_{T^-,test} = 1$			N/A
p_{res}	Production rate of resource, either as bulk or by cells	O_2	0.11	min^{-1}	Equation 2.11, Equation 2.12
		$test, max$	5×10^{-7}	$\text{min}^{-1}\text{cell}^{-1}$	
$p_{test,min}$	Production rate of $test$ under abi therapy	1×10^{-7} $\text{min}^{-1}\text{cell}^{-1}$			Equation 2.13
$\mu_{res,i}$	Uptake of resource res by cell type i	O_2	T^+	1.63×10^{-6} $\text{min}^{-1}\text{cell}^{-1}$	(Hail et al., 2010), Equation 2.12
			T^p	1.63×10^{-6} $\text{min}^{-1}\text{cell}^{-1}$	
			T^-	1.04×10^{-6} $\text{min}^{-1}\text{cell}^{-1}$	
		$test$	T^+	2.34×10^{-8} $\text{min}^{-1}\text{cell}^{-1}$	
			T^p	6.00×10^{-8} $\text{min}^{-1}\text{cell}^{-1}$	
			T^-	0 $\text{min}^{-1}\text{cell}^{-1}$	

Continued on next page

Parameter	Description	Value(s)	Source(s)
λ_{res}	Decay rate of resource res	O_2: 0.100 min^{-1} $test$: 0.004 min^{-1}	(Jain et al., 2011)
$ll_{res,i}$	Lower limit/threshold level of resource res for carrying capacity of cell type i	$\in [0, 1]$	N/A
$ul_{res,i}$	Upper limit/saturation level of resource res for carrying capacity of cell type i	$\in [0, 1]$	N/A
Supplementary Parameters			
τ_d	Doubling time of cell type i	T^+: 34 hr T^p: 40 hr T^-: 25 hr	(ATCC, 2021)
y_i^*	Equilibrium value of cell number in absence of competition	10000	assumed
$y_{i,dtx}^*$	Equilibrium value of cell number in absence of competition under dtx therapy	T^+: $0.30 \times y_i^*$ T^p: $0.30 \times y_i^*$ T^-: $0.15 \times y_i^*$	(Morikawa et al., 2012)
res^*	Equilibrium/Tissue levels of resource with one cell type present	O_2: 2.5 mmHg $test$: 3.74 pmol/g tissue	(Stewart et al., 2010),(Titus et al., 2005)
$test_{abi}^*$	Equilibrium/Tissue levels of testosterone with only T^p cell type present under abi therapy	$0.1 \times test^*$	(Acharya et al., 2012)

Table 2.1: Table of all parameters

Chapter 3

Pairwise Interactions and Competition outcomes

3.1 T^p - T^-

A set of initial runs was carried out to test the extent to which cell type-specific doubling times affected the competitive outcomes between T^p and T^-, from which the following observations were made:

1. Only when T^p is not severely testosterone limited (ul_{test,T^p} is low), T^p can coexist with or outcompete T^- as shown in Figure 3.1. In every other case, T^- drives T^p to extinction.

2. These competitive outcomes are also dependent on the initial proportion of T^p, all the other parameters being the same as shown in Figure 3.1.

3. When T^- is strongly oxygen-limited ($ll_{O_2,T^-} \geq 0.6$) but T^p is also limited by testosterone. In this case, T^- wins out eventually as oxygen levels rise faster than testosterone through the external supply term, p_{O_2} as shown in Figure 3.2.

4. When T^- is oxygen limited but with poor oxygen production (lower p_{O_2}), T^p is able to drive T^- to extinction as T^p can grow and consume enough oxygen to keep the oxygen levels below those required for T^- to grow as shown in Figure 3.2.

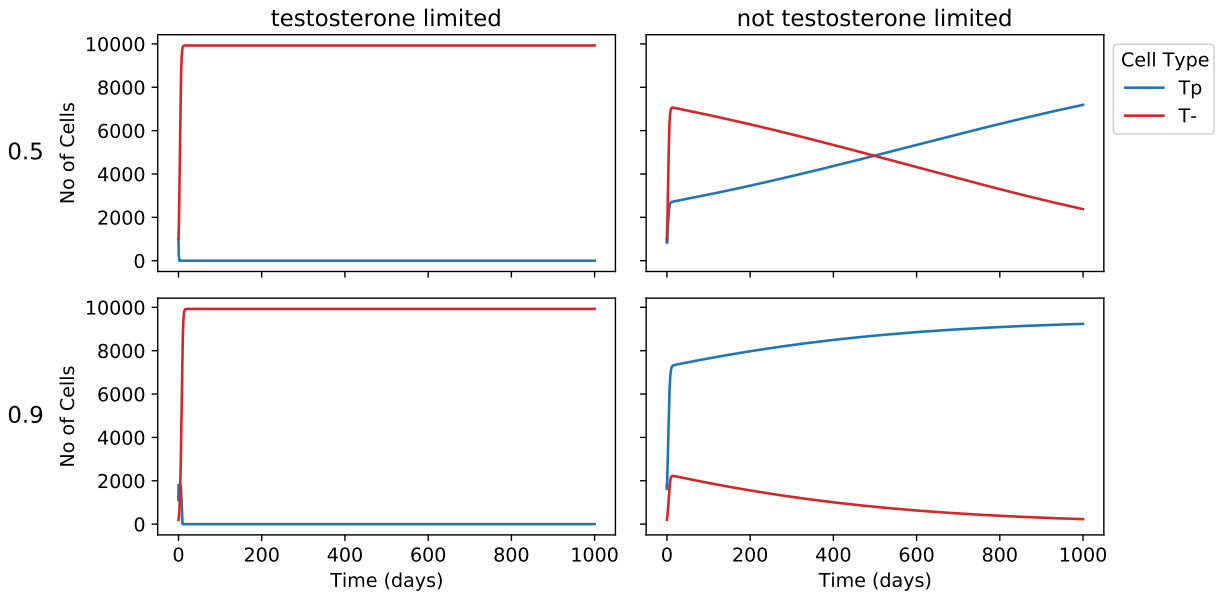


Figure 3.1: Pairwise $T^p - T^-$ time-series, when T^p is testosterone limited and not testosterone limited (columns) and at different initial proportions of T^p (rows). T^p is testosterone limited at $ul_{test,T^p} = 0.5$ and not testosterone limited at $ul_{test,T^p} = 0.1$.

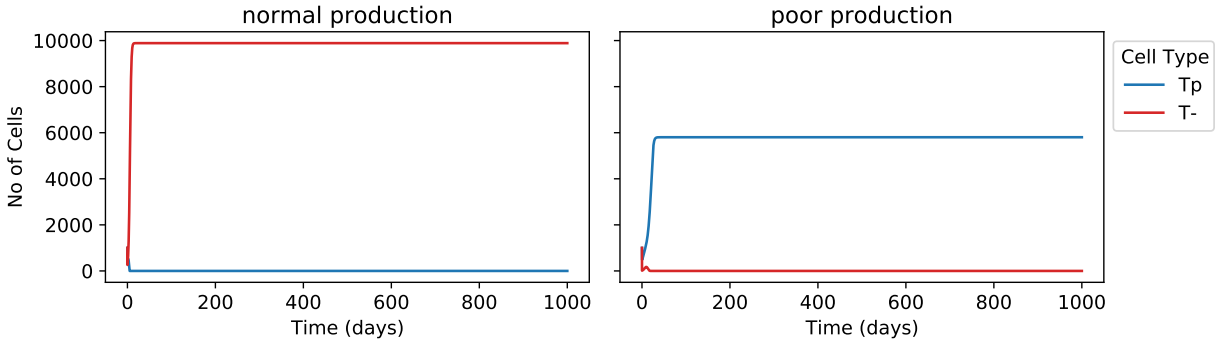


Figure 3.2: Pairwise $T^p - T^-$ time-series, when T^- is oxygen limited and at different oxygen production (column). T^- is oxygen limited at $ll_{O_2,T^-} = 0.6$ and T^p is testosterone limited at $ul_{test,T^p} = 0.5$. The normal and poor production of oxygen are 0.11 and 0.0675 min^{-1} respectively

Taken together, these provide the first proof-of-concept that competitive outcomes can be controlled through the interaction of cell types with the resources in the environment.

Building on these initial observations, a brute force parameter space exploration was done over a large combination of parameters. The entire dataset is large and a few generalised observations collated from these data have been listed below.

1. T^- drives T^p to extinction when $ll_{O_2,T^p} \geq 0.6$, regardless of the other parameters; in other words, T^p should not be limited by oxygen if it is to compete with T^-.

2. T^- drives T^p to extinction when $ll_{test,T^p} \geq 0.2$, regardless of the other parameters; in other words, T^p needs to be able to grow even on the smallest amount of testosterone to compete with T^-.

3. T^- drives T^p to extinction when $ul_{test,T^p} \geq 0.3$ and $ll_{O_2,T^-} \leq 0.4$ but not when $ll_{O_2,T^-} \geq 0.6$; in other words, T^p shouldn't be testosterone limited when T^- is not oxygen limited to be to compete with T^-. The ul_{test,T^p} required for T^p to not go extinct also increases with increased ll_{O_2,T^-}, that is, T^p can afford to be more testosterone limited as T^- becomes more oxygen limited.

These observations allow us to then define levels of resource limitation for each cell type, which represent the competitive strategy employed by that cell type. We fix three levels each of T^p *test* limitation: no, moderate and severe corresponding to $ul_{test,T^p} = 0.1, 0.3, 1$ respectively and three levels each of T^- O_2 limitation: low, high and severe corresponding to $ll_{O_2,T^-} = 0, 0.6, 0.8$ respectively. As found earlier, T^p goes extinct at any level of oxygen limitation and therefore offers no scope for exploration along this axis. We also explore two levels of O_2 production: normal and poor, corresponding to $p_{O_2} = 0.11, 0.0675$ min^{-1} respectively. Pairwise competitive runs were done over all combinations of these (as shown in Table 3.1) with varying initial cell seeding.

O_2 **production**	T^- O_2 **limitation**	T^p *test* **limitation**
normal	low	no
normal	low	moderate
normal	low	severe
normal	high	no
normal	high	moderate
normal	high	severe
normal	severe	no
normal	severe	moderate
normal	severe	severe
poor	low	no
poor	low	moderate
poor	low	severe
poor	high	no
poor	high	moderate
poor	high	severe
poor	severe	no
poor	severe	moderate
poor	severe	severe

Table 3.1: Table of cases for T^p - T^- pairwise

The following were observed from the cases as visualised in Figure 3.3:

1. Coexistence is observed only when there is no or moderate limitation of testosterone for T^p and low limitation of oxygen for T^-. For low T^p initial seeding, T^- dominates over T^p and causes it to go extinct, but as T^p initial seeding increases the favour shifts towards T^p.

2. T^- causes T^p to go extinct for all initial seedings when T^p is severely testosterone limited. Even with a high initial seeding advantage, T^- grows, overtakes T^p and eventually causes T^p to go extinct. T^- also goes extinct in this case if it is limited by oxygen under poor oxygen production. Despite this, T^p is weighed down by both the testosterone limitation and density-dependent competition of the remaining T^- cells and goes extinct as a result.

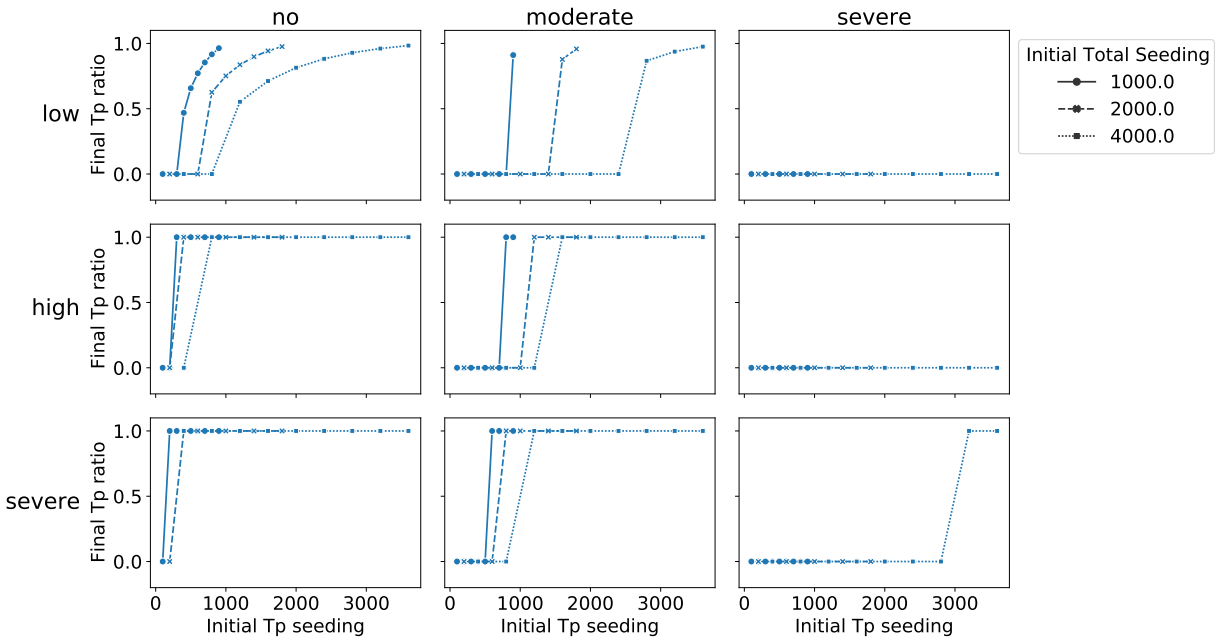
3. The outcome switches from T^p going extinct to T^- going extinct for higher T^p initial seeding when T^- is highly limited by oxygen under either poor or normal oxygen production and when T^- is severely limited by oxygen under normal oxygen production.

Similar to the cases with coexistence, for low T^p initial seeding, T^- dominates over T^p and causes it to go extinct, but as T^p initial seeding increases the favour shifts towards T^p. However, in this case the oxygen levels don't go above the levels required for T^- to grow before it goes extinct and only T^p remains.

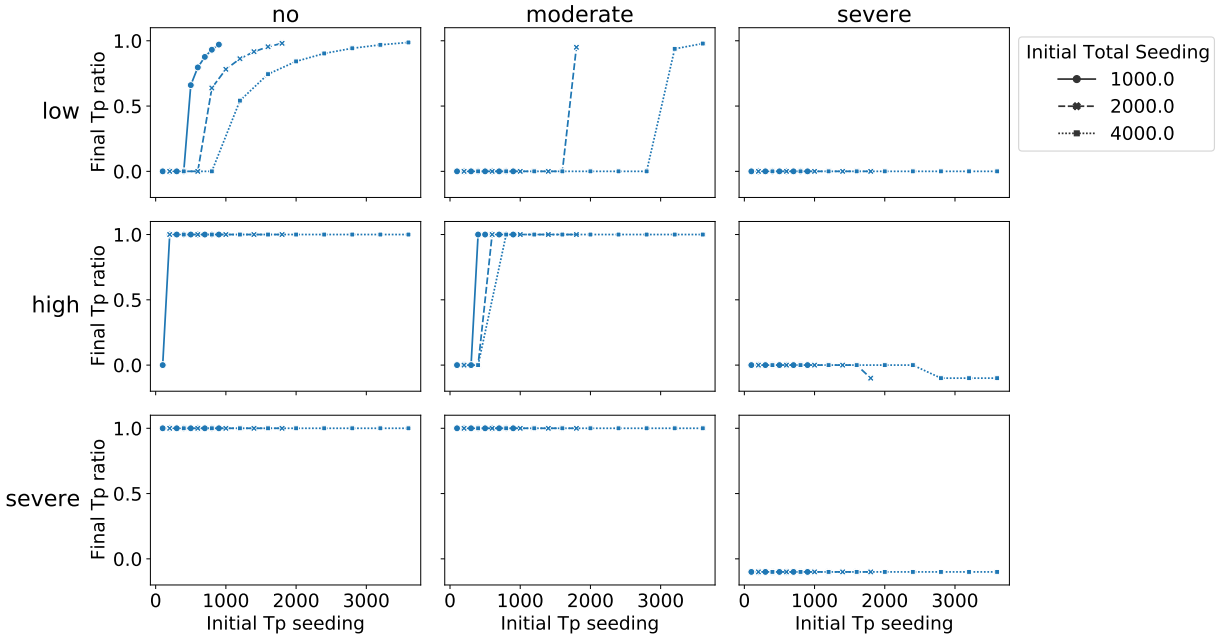
4. T^- goes extinct for all initial seedings when it is severely oxygen limited under poor oxygen production. The oxygen limitation on T^- is too high and the oxygen levels never reach the levels required for a non-zero growth for T^-.

5. Additionally, total population size has a weaker effect than initial proportion for the dynamics and outcomes for each particular case.

These observations expand and broaden the scope of the earlier finding that cell-intrinsic doubling times have a relatively marginal effect on competitive outcome; with the data in Figure 3.3, it is possible to pinpoint which resource conditions favour each cell type based on their relative limitation strengths for that resource.



(a) normal production



(b) poor production

Figure 3.3: Final T^p ratio of pairwise $T^p - T^-$ runs under different cases. Subfigures: O_2 Production, Rows: T^- O_2 limitation, Columns: T^p *test* limitation. Note: Ratio = -0.1 is used when both cell types go extinct.

3.2 T^+ - T^p

The initial runs for this pair involved changing both the upper and lower limits of the resource response function together as a way of sampling resource limitation conditions coarsely before a more detailed exploration. The following observations were made based on these results, represented in Figure 3.5 and Figure 3.4:

1. Both T^+ and T^p are limited by both oxygen and testosterone, and compete for both resources. As with the other pair, strength of limitation for any particular resource can be modulated through the corresponding upper and lower thresholds.

2. When T^p is limited by testosterone more than T^+ ($ul_{test,T^p} > ul_{test,T^+}$), T^+ can consume and grow on the limited testosterone present, and this is enough for the density-dependent competition to drive T^p to extinction. Without T^p to provide testosterone, T^+ subsequently goes extinct.

3. When T^p is weakly limited by testosterone relative to T^+ ($ul_{test,T^p} \leq ul_{test,T^+}$), both cells coexist. Due to weaker testosterone limitation, T^p can grow faster initially and secrete enough testosterone for T^+ without being negatively affected by T^+. This is visualised in Figure 3.4.

4. When both are severely testosterone limited but not oxygen limited, T^p causes T^+ to go extinct. However, in a special scenario when both are oxygen limited with T^+ being more limited, coexistence is observed. A balance of sort is achieved here, where, in the initial period of low oxygen, T^p can grow more than T^+ and secrete enough testosterone to sustain both population but doesn't grow as much as to drive T^+ to extinction. This is visualised in Figure 3.5.

As with $T^p - T^-$, cell type competitive strategies were encoded in terms of levels of resource limitation for each cell type. Three levels each of T^p *test* and T^+ *test* limitation: no, moderate and severe corresponding to $ul_{test,T^i} = 0.1, 0.3, 1$ respectively, Three levels each of T^p O_2 and T^+ O_2 limitation: low, moderate and severe corresponding to $ll_{O_2,T^i} = 0, 0.4, 0.8$ respectively were considered and pairwise competitive runs were done over some combinations of these (as shown in Table 3.2) with varying initial cell seeding.

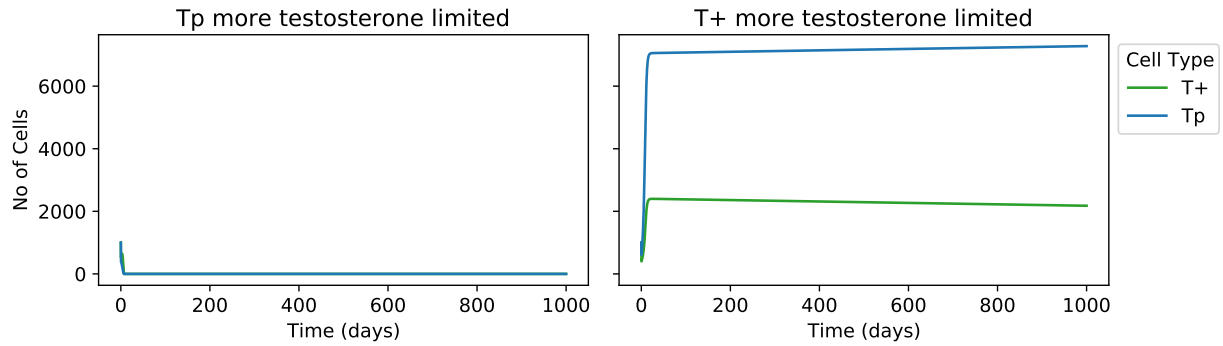


Figure 3.4: Pairwise $T^+ - T^p$ time-series, when T^p is more testosterone limited than T^+ and when T^+ is more testosterone limited than T^p. T^p is more limited testosterone limited at $ul_{test,T^+} = 0.3, ul_{test,T^p} = 0.5$ and T^+ is limited more at $ul_{test,T^+} = 0.5, ul_{test,T^p} = 0.3$.

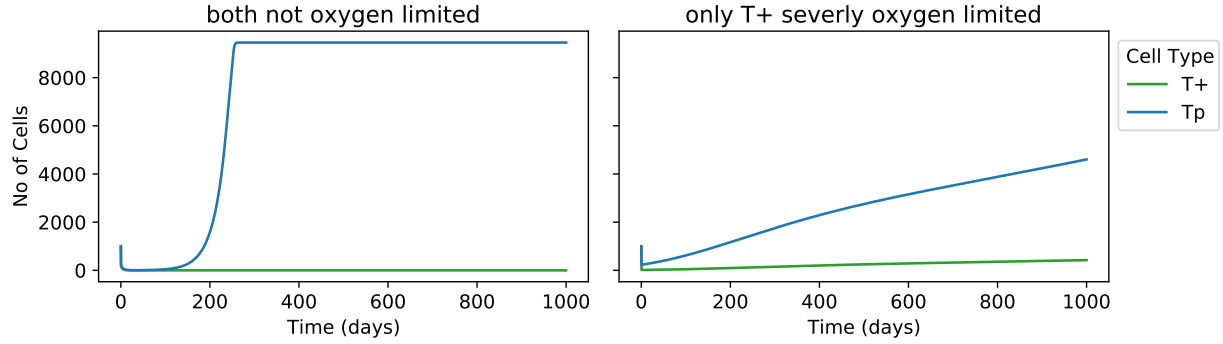


Figure 3.5: Pairwise $T^+ - T^p$ time-series, when both cell types are testosterone limited and not oxygen limited at $ll_{O_2,T^+} = 0.0, ll_{O_2,T^p} = 0.0$ and T^+ is oxygen limited and T^p moderately at $ll_{O_2,T^+} = 0.6, ll_{O_2,T^p} = 0.4$.

Only two levels of limitations were considered for each resource when combinations of both O_2 or *test* limitations were done to reduce the number of combinations for better interpretability. Similarly, different levels of O_2 or *test* production is not considered in these cases for the same reason. Production terms will ultimately affect resource availability, and adjusting the response function of the cell should be qualitatively equivalent to reducing actual resource concentrations.

T^+ O_2 **limitation**	T^p O_2 **limitation**	T^+ *test* **limitation**	T^p *test* **limitation**
low	low	no	no
low	low	no	moderate
low	low	no	severe
low	low	moderate	no
low	low	moderate	moderate
low	low	moderate	severe
low	low	severe	no
low	low	severe	moderate
low	low	severe	severe
low	moderate	no	no
low	moderate	no	moderate
low	moderate	moderate	no
low	moderate	moderate	moderate
low	severe	no	no
moderate	low	no	no
moderate	low	no	moderate
moderate	low	moderate	no
moderate	low	moderate	moderate
moderate	moderate	no	no
moderate	moderate	no	moderate
moderate	moderate	moderate	no
moderate	moderate	moderate	moderate
moderate	severe	no	no
severe	low	no	no
severe	moderate	no	no
severe	severe	no	no

Table 3.2: Table of cases for T^+ - T^p pairwise

The following were observed from the different cases. For better visualisation, the figures are divided into testosterone limitations in Figure 3.6a, oxygen limitations in Figure 3.6b and combinations of the limitations in Figure 3.6c.

1. Severe limitation of either oxygen or testosterone for T^+ relative to T^p causes it to go extinct. In a special case, when T^p numbers are high enough to produce an excess of testosterone, a small fraction of T^+ survives regardless of the strength of T^+ test limitation. Conversely, when neither resource is limiting, coexistence occurs at all seeding densities and proportions of T^p, which suggests that competitive exclusion of either cell type is strongly dependent on environmental conditions and resource limitation. When T^+ is moderately oxygen limited relative to T^p, T^+ can coexist at low initial density of T^p but goes extinct at higher initial densities.

2. T^p is driven to extinction in every case where T^p limitation of either oxygen or testosterone is more severe relative to T^+ limitation of the same resource. Extinction of T^p then leads to extinction of T^+ trivially. Such is the case for the most part with moderate limitation of testosterone for T^p relative to T^+. However, this T^p extinction is seen to be rescued for higher initial density of T^p relative to T^+ as this allows the former to overcome competition, leading to coexistence.

3. In very broad terms, coexistence is more common when the strength of limitation of either resource is the same for both cell types-these are the main diagonals in Figure 3.6a and Figure 3.6b. Generally, increasing the relative proportion of T^p gives it a competitive edge presumably by increasing net availability of testosterone in the system. However, under high testosterone limitation for both cell types, a larger T^p proportion is marginally detrimental to T^p success possibly due to density-dependent intraspecific competition.

4. With moderate limitation of oxygen for T^p relative to T^+, T^+ still requires testosterone from T^p for survival which could weaken the growth inhibition of T^p, despite the lower oxygen limitation of T^+ compared to T^p. Interestingly, this is also the case with coexistence at lower final T^p proportions than any other case. Coexistence here is therefore driven by the dependence on T^p by T^+ for testosterone, which overrides any advantage from a better oxygen use strategy.

5. Coexistence is also observed when T^+ is moderately testosterone limited relative to T^p. However, in this case, a lower initial proportion of T^p favours T^p and leads to a

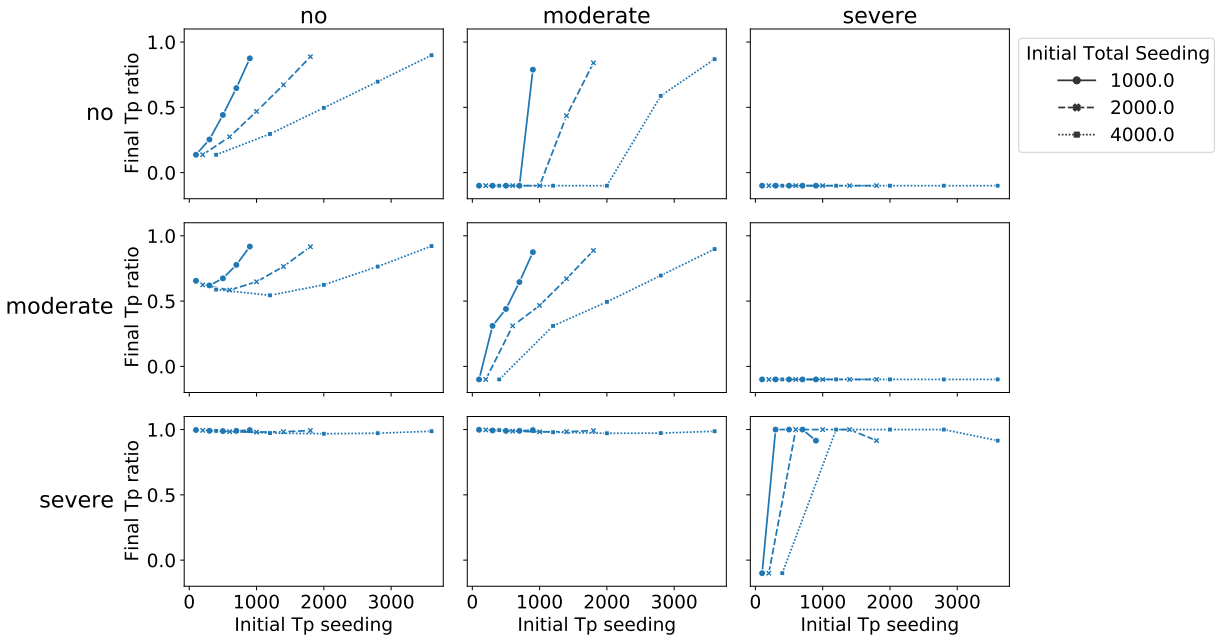
dip in the plot. At a low initial proportion of T^p, T^+ being limited by testosterone dies out until sufficient testosterone is established and this might give an advantage for T^p to establish a larger population before T^+ has the capacity to compete.

6. The behaviour of the system is very similar if the resource limitation is symmetric across the two cell types. This can be seen from the first and last columns or from the first and last rows of Figure 3.6c. Although, with the higher testosterone limitations of T^p, a higher T^p initial seeding is required to have T^p overcome suppression by T^+. Additionally, when T^p is moderately limited by oxygen relative to T^+, the higher testosterone limitation of T^+ leads to higher T^p required for the testosterone.

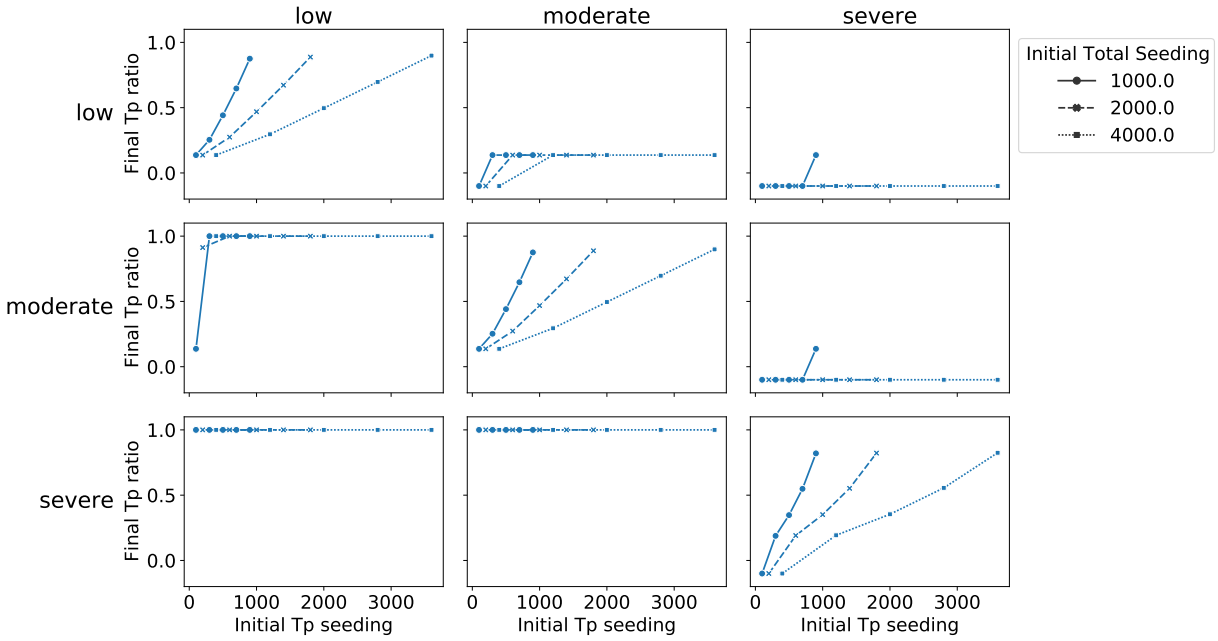
7. When both testosterone and oxygen are moderately limiting for a cell type relative to the other, the combined overall limitation is severe and that particular cell type is driven to extinction similar to when only one resource was severely limiting.

8. When T^p is moderately limited by oxygen relative to T^+ and T^+ moderately limited by testosterone relative to T^p, a balance is achieved. T^+ can outcompete T^p due to the excess oxygen but soon is limited by testosterone and has to allow a sizeable population of T^p to grow to maintain the required testosterone levels. However, with the inverse case where T^p is testosterone limited and T^+ is oxygen limited, the outcomes are unstable and it switches from T^p driven to extinction at low initial proportion to T^+ going extinct for higher initial proportion.

9. Additionally, total population size has a weaker effect than initial proportion for the dynamics and outcomes for each particular case.

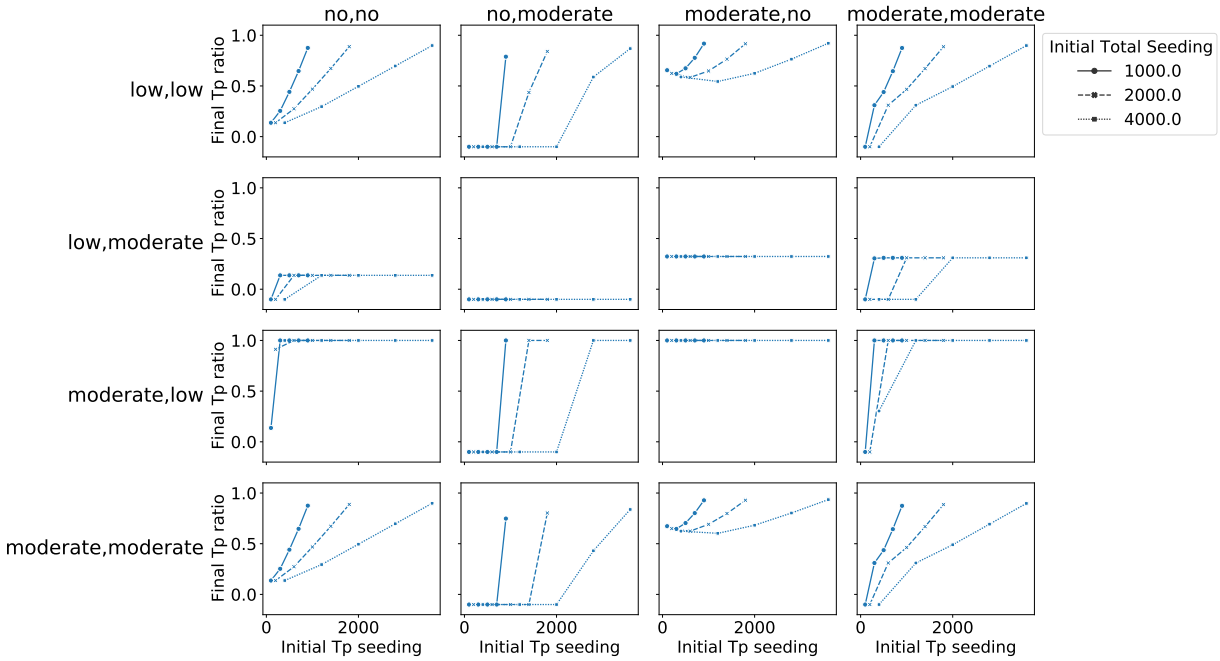


(a) *test* limitations. Columns: T^p *test* limitation, Rows: T^+ *test* limitation.



(b) O_2 limitations. Columns: T^p O_2 limitation, Rows: T^+ O_2 limitation.

Figure 3.6: (Continued on next page)



(c) Combination of *test* and O_2 limitations. Columns: T^+, T^p *test* limitations, Rows: Columns: T^+, T^p O_2 limitations.

Figure 3.6: Final T^p ratio of pairwise $T^+ - T^p$ runs under different cases. Note: Ratio = -0.1 is used when both cell types go extinct.

Chapter 4

All cell types-interactions and competition outcomes

With all three cell types, the number of combinations and permutations increase combinatorially which restricts the tractability of giving different strategies to each cell type as has been done with the pairwise simulations. We are therefore starting with a simpler case of the same strategy for all the three cell types. The strategies are still defined in terms of levels of resource limitations, with the distinction that now, the level of each strategy is the same for all the three cell types. The values of lower and upper limits corresponding to each level are given in Table 4.1.

Resource	**Limitation**	$ll_{res,i}$	$ul_{res,i}$
Oxygen	no	0.0	1.1
	low	0.0	1.0
	moderate	0.4	0.1
Testosterone	no	0	0.1
	moderate	0	0.3

Table 4.1: Table of limits corresponding to limitations for different resources of all three cell-types

Competitive runs were done over all the six combinations of limitations for two different ratios of seeding and three initial total seedings. The following were observed from the different cases as visualised in Figure 4.1. The time-series of the same is given in Figure A.1.

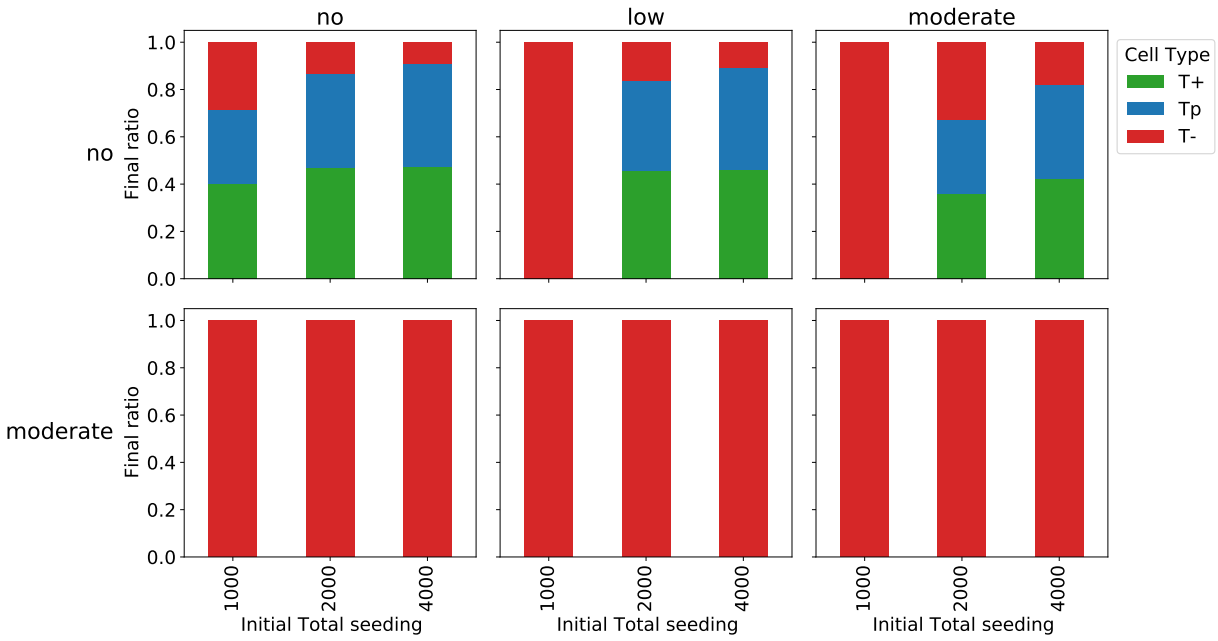
1. Moderate limitation of testosterone leads to stronger interspecific competition relative to intraspecific competition as T^p and T^+ numbers cannot increase enough to produce self-inhibition. In these cases, inhibition by T^- is much stronger and they are driven to extinction, as opposed to when T^p and T^+ coexisted when seeded without T^-. However, consistent with the $T^p - T^+$ results, coexistence can be recovered between all three cell types when T^p is seeded at a much higher proportion than the other two, presumably because of higher net available testosterone in the system. Therefore, when testosterone is a strongly limiting resource, it also leads to strong positive dependence on T^p density for coexistence.

2. No limitation of testosterone leads to weaker interspecific competition relative to intraspecific competition, but only above a threshold proportion of T^p that is required to produce a minimum amount of testosterone for survival. Again, we see that self-production of testosterone by T^p leads to progressively lower limitation of the hormone across runs with increasing number of T^p cells, as well as within a given run with increasing time. Additionally, in the moderate testosterone limitation cases, it can be seen that lower oxygen limitation reinforces this positive feedback on T^p further, leading to coexistence that is further biased towards the $T^p - T^+$ pair.

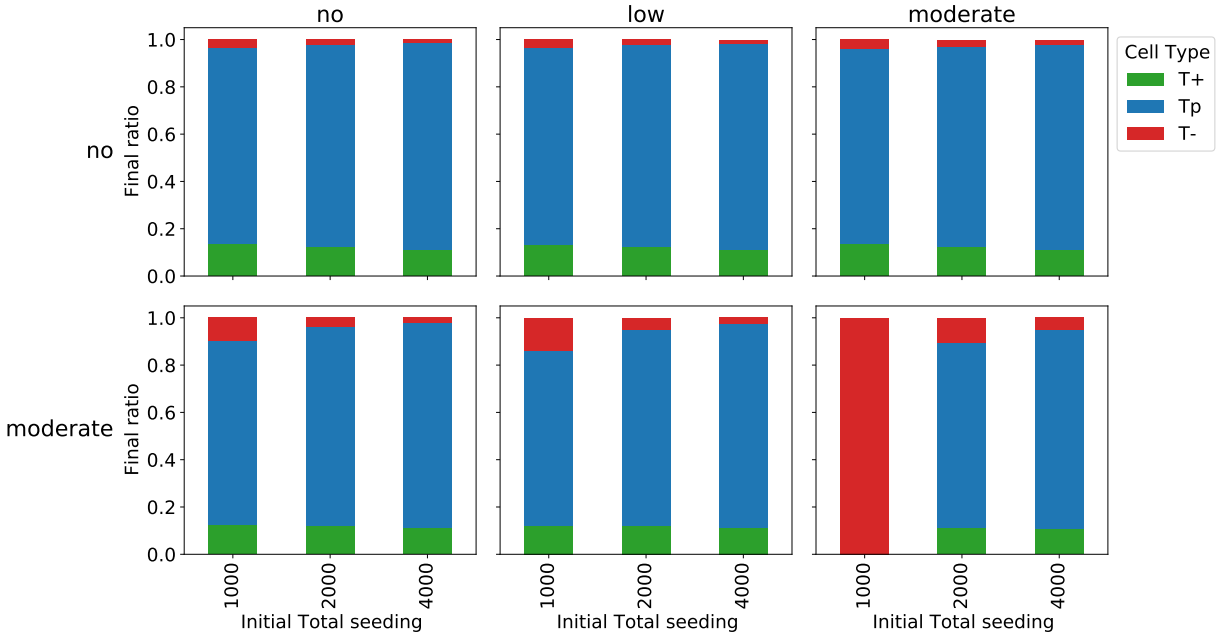
3. Testosterone then acts as a private resource and the primary limitation between T^p and T^+ that serves to produce positive feedback above some minimum test concentration, thus leading to coexistence between all three cell types even though T^- has a significantly shorter doubling time.

Taken together, the resource limitation space can be divided into three broad zones of cell type coexistence in the system. At high limitations of testosterone, the growth of T^p is so strongly inhibited that coexistence is rare, independent of the availability of oxygen; indeed, the addition of oxygen limitation only drives T^p down further and makes coexistence even more remote. At the other extreme of low limitation of testosterone, T^p growth inhibition is relieved and the positive feedback from T^p-produced testosterone is strong enough that oxygen limitations become relatively less important for coexistence. This is abundantly clear

from when T^p is seeded at a much higher density than the other cell types [Figure 4.1b]. Oxygen limitations are seen to impact coexistence only when testosterone availability is above some minimum threshold required for $T^p - T^+$ growth, but not so high as to saturate all growth. In this range of resource availability, within the limits of the parameter values tested here, continuous responses to change in resource concentration are observed, and the outcomes of competition are pushed either towards coexistence or extinction of T^p and T^+ by the oxygen limitation. Therefore, across these three zones, limitations of oxygen seem to have a subordinate impact on competition and instead supplement the competition imposed by testosterone; where oxygen limitation does affect competitive outcomes, it does so in the same direction as testosterone limitation.



(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1



(b) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1

Figure 4.1: Final ratio of all cell types under different oxygen limitation (columns), testosterone limitation (rows) and initial seeding proportions (subfigures).

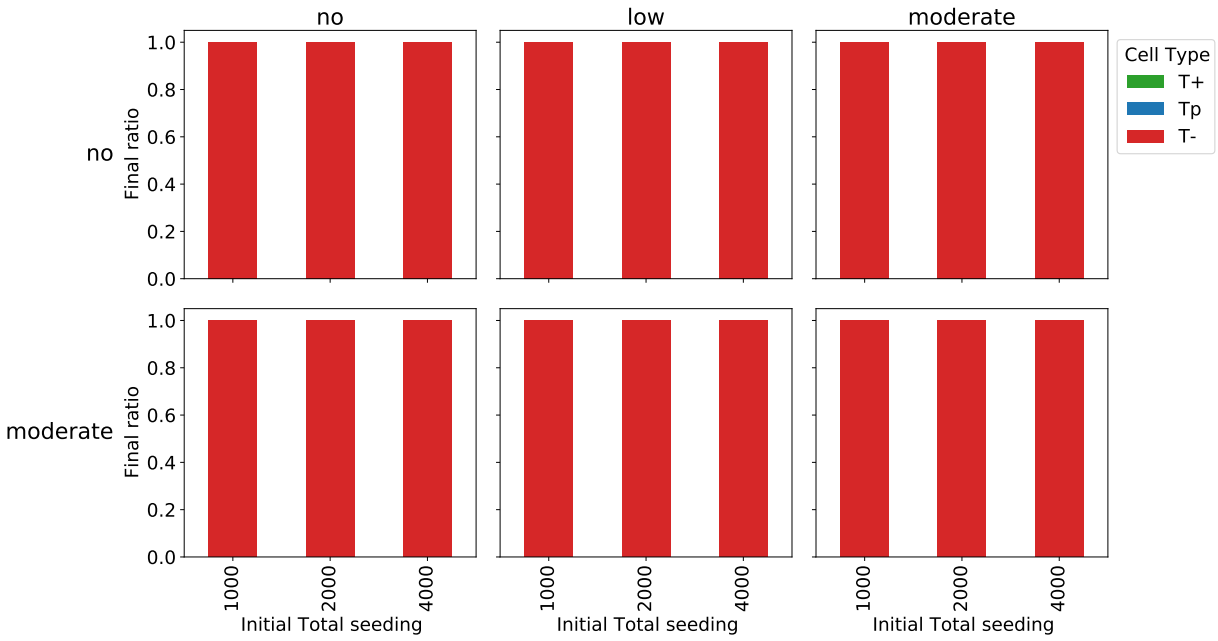
Chapter 5

All cell types-therapy outcomes

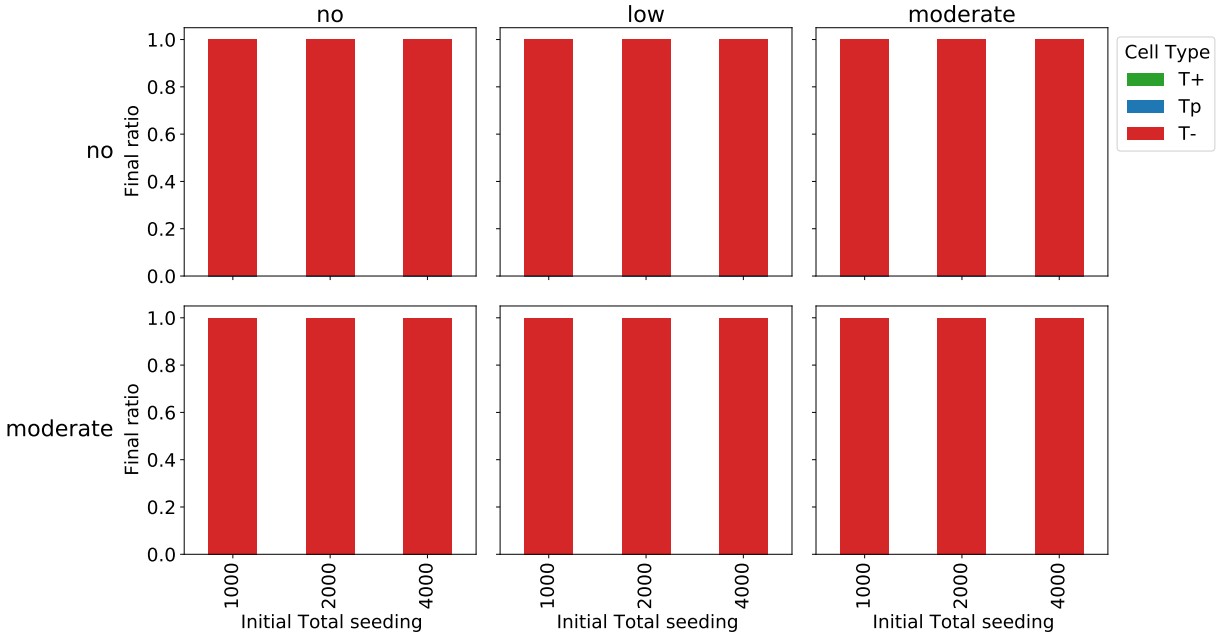
5.1 Standard of care (SOC)

Under standard-of-care, the drug is applied from the beginning of the simulation at the maximum tolerated dose for all the cases explored in the previous chapter. In our model, this is simulated as a permanently reduced rate of testosterone secretion by T^p.

We observe from Figure 5.1 that T^+ and T^p go extinct in all the cases, regardless of the limitations of either resource, the seeding proportion or initial total seeding. This can be rationalised based on the results from the previous chapter, where testosterone limitation had a similarly drastic effect on coexistence, or lack thereof, between the three cell types. In this case, with therapy, the nearly immediate elimination of T^+ and T^p due to insufficient levels of testosterone leads to competitive release of T^-, and the total population reaches its maximum effective carrying capacity. Given that abiraterone acts by suppressing testosterone secretion, an entire population of T^- cells would be fully unresponsive to abiraterone treatment, and further treatment cannot lead to any reduction of cell number.



(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1



(b) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1

Figure 5.1: Final ratio of all cell types under standard-of-care under different oxygen limits of T^- (columns), oxygen efficiencies of T^+, T^p (rows) and initial seeding proportions, initial total seeding (subfigures).

5.2 Adaptive Therapy (AT)

Implementation of adaptive therapy (AT) is based on thresholds of population size; the On threshold is the size above which therapy would be applied and the Off threshold is the size below which therapy would be withdrawn. A range of such size thresholds were tried out for the case where testosterone is not limiting and oxygen has low limitation. For the purposes of this model, abiraterone therapy thresholds were defined in terms of the population sizes of the hormone-dependent subpopulations alone, T^+ and T^p. There are some indications from the literature that distinguishing between hormone-dependent and -independent fractions of prostate cancer cells may be possible clinically based on levels of specific prostate-specific antigen (PSA) types secreted by each (Takahashi et al., 1999).

The following general observations could be made based on our exploration of population size thresholds, some of which are visualised in Figure 5.2.

1. In the AT modelling literature, a 50% rule is commonly-used where therapy is applied above the initial population and withdrawn below 50% of that. In our exploration, we found that for low threshold sizes, the T^p and T^+ populations are so low that competitive inhibition by T^- drives them to extinction. This low threshold size was also found to include the population size range where the 50% rule would operate, which casts some doubt on the effectiveness of this threshold.

2. It follows directly from above that a higher threshold would be better for T^p and T^+ to survive the competition by T^- as well as to suppress T^-, while increased effectiveness of a higher threshold has also been shown elsewhere (Hansen & Read, 2020). However, increasing the threshold close to the effective carrying capacity eventually leads to no therapy being applied for the entire simulation duration as the population sizes never cross the On threshold. While there may be some grounds for a clinical decision not to apply any treatment, such a situation is not suited for an exhaustive theoretical study of the factors affecting AT. The threshold of On:6000 and Off:4000 was therefore chosen for further exploration as a reasonable middle ground between competitive release and no therapy.

3. As mentioned earlier, only T^p and T^+ cell types were considered for the thresholds for therapy. For the sake of completeness, the case where all the three cell types are

considered for the thresholds for therapy was also tried out as visualised in Figure A.3. However, all the cases led to competitive release in the initial few days, suggesting that total population size makes for a poorer cue for AT application and withdrawal.

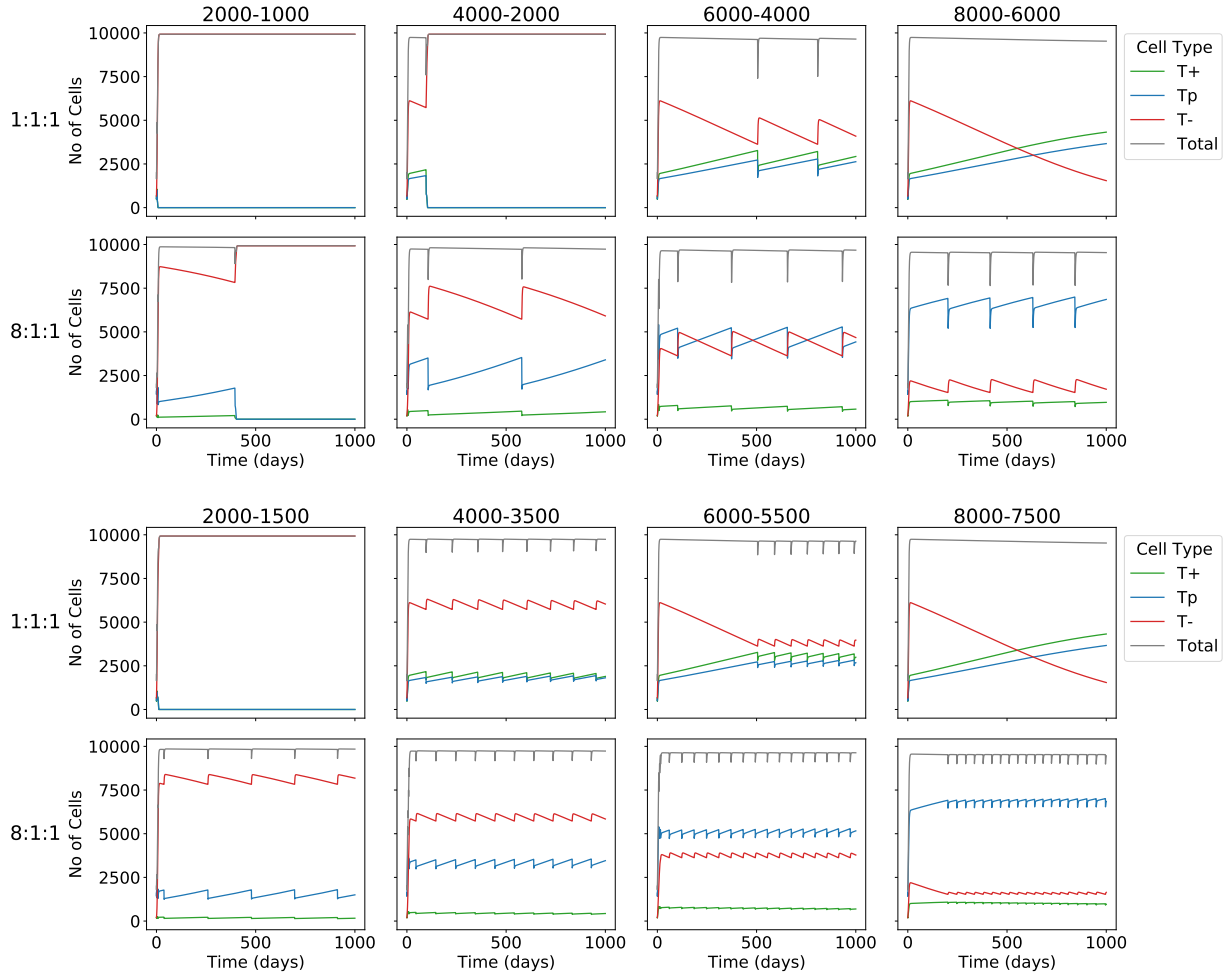


Figure 5.2: Standardisation of threshold for adaptive therapy, Columns: $On-Off$ threshold, Rows: $T^p : T^+ : T^-$ Seeding

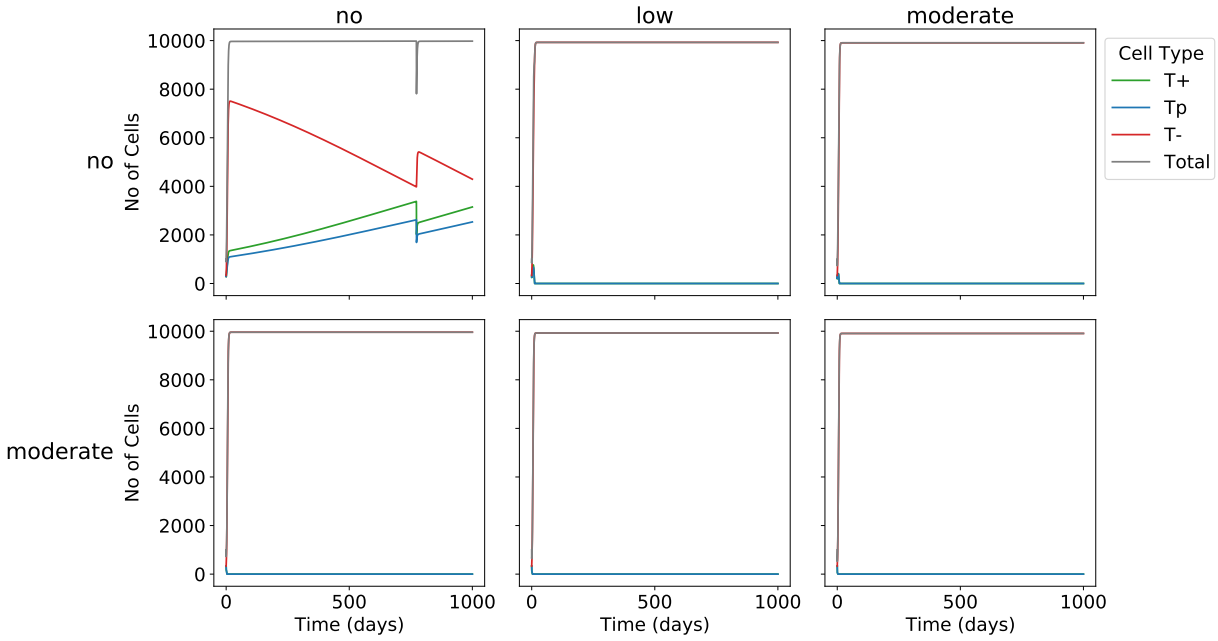
5.2.1 Without delay

With fixed On and Off thresholds of 6000 and 4000 respectively, AT was then applied systematically to every combination of resource limitation studied in the previous section. The results from these different cases are summarised below and visualised in Figure 5.3.

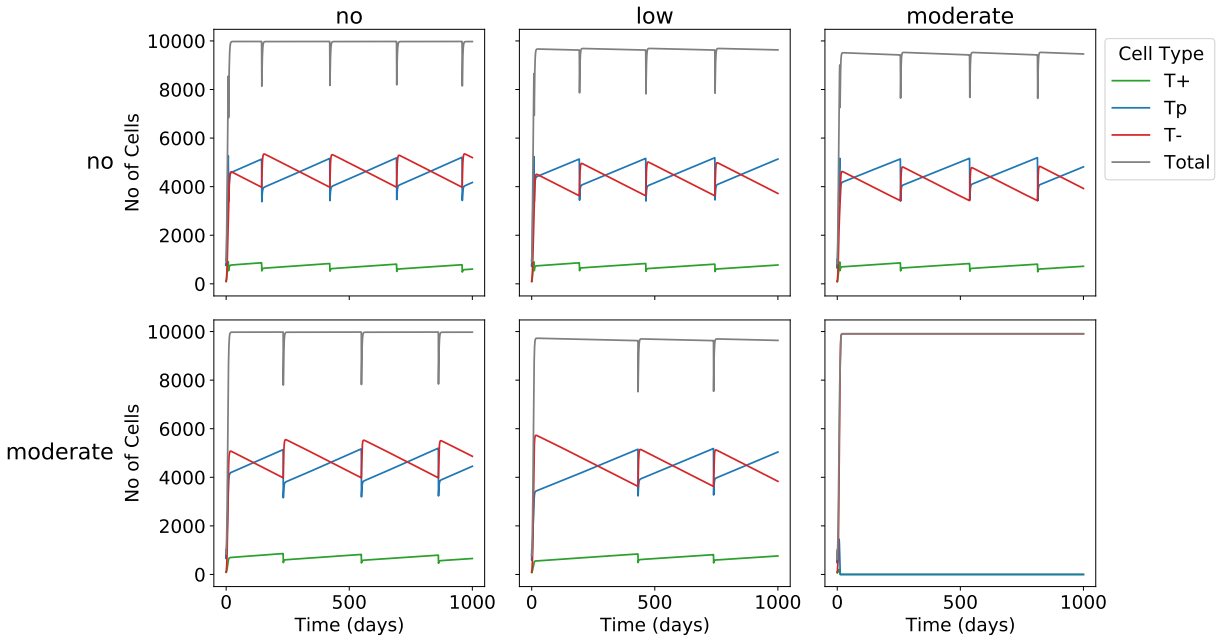
1. Tumours with higher numbers of T^p and T^+ would be more responsive to abiraterone and hence more treatable. Coexistence is of importance here as extinction of T^p and T^+ would lead to no response.

2. For cases when testosterone is moderately limiting and testosterone levels below the requirement of T^p and T^+, these two cell types go extinct just by the competition from T^- and produce no response from abiraterone naturally. Therefore, abiraterone efficacy depends strongly on T^p seeding densities and total population, as with coexistence.

3. In cases where coexistence is achieved, the T^- cells quickly replace the space left by the dead T^p and T^+ cells on applying therapy. In periods of no therapy, T^p and T^+ cells compete with and replace the T^- cells, but are soon met with therapy as they cross the On threshold. The total population size therefore remains high for most of the simulation, except when abiraterone is being applied where the T^p population falls sharply. Despite this dip, the total population size recovers rapidly once therapy is withdrawn although there is some indication that total population size subsequently declines gradually until the next application, as T^- cells are driven down by competition. Net decrease in the total population size itself, across multiple oscillations, is only seen in some combinations of resource limitation. In particular, this happens when testosterone is limiting (moderate limitation case) and/or the tumour population is still highly responsive to abiraterone (an active and considerable proportion of T^p in the population), raising the possibility that even though AT, with a fixed window of treatment, outperforms SOC across the board, its effectiveness is modified strongly by the ecological state of the tumour population, in terms of cell type proportions and resource limitations.

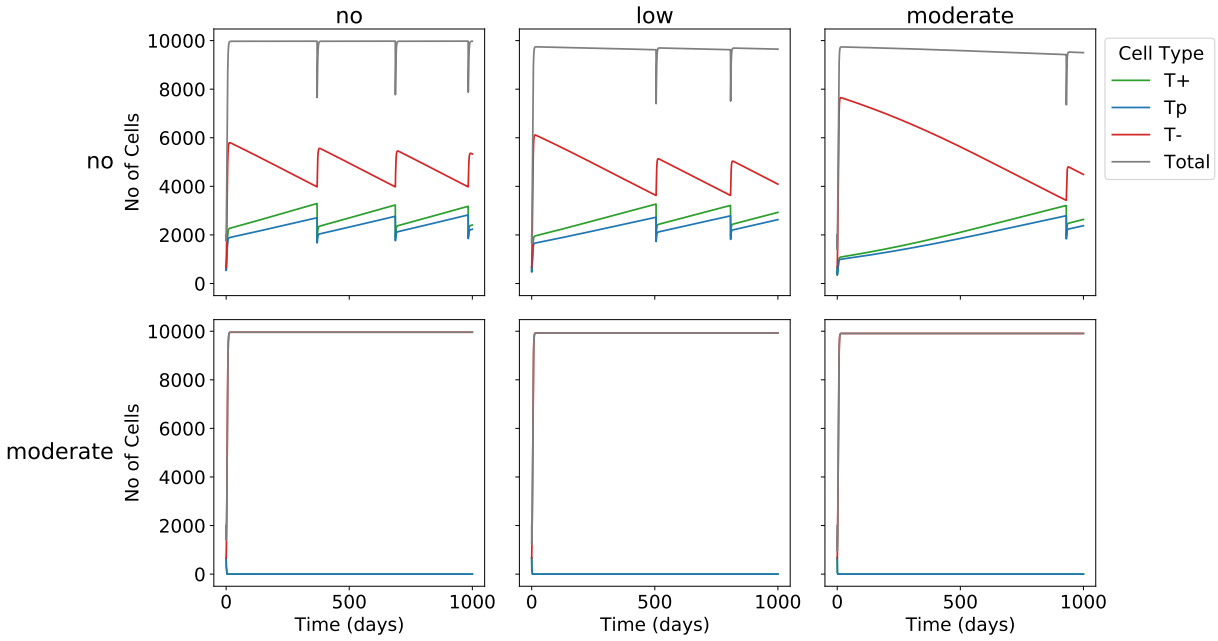


(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 1000

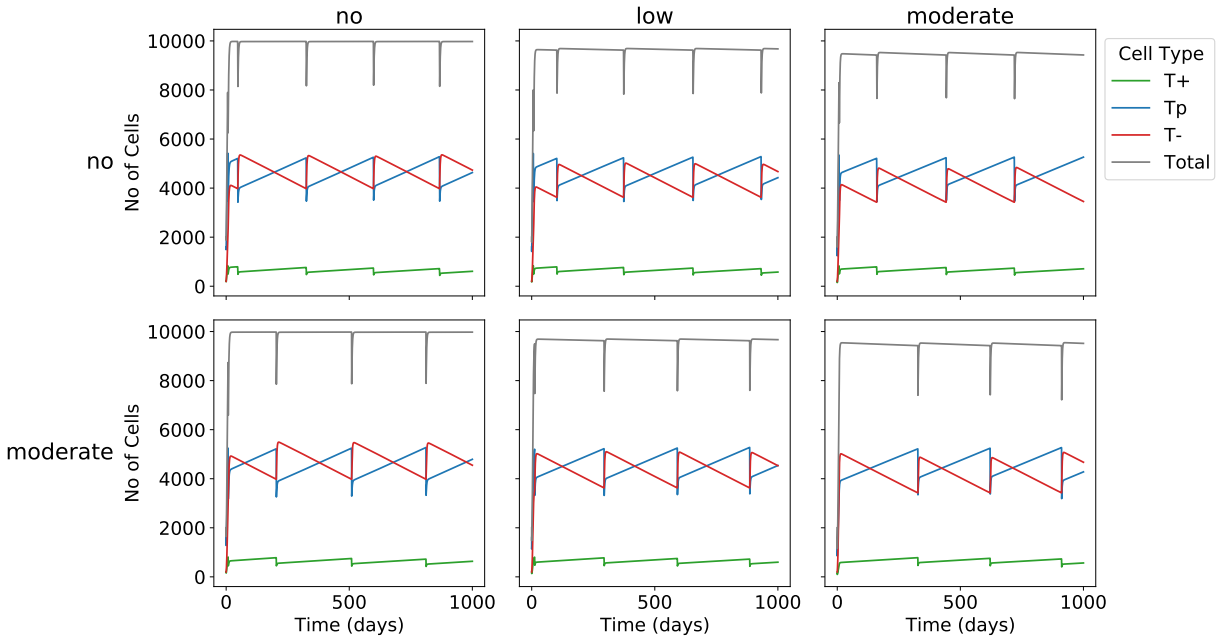


(b) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 1000

Figure 5.3: (Continued on next page)

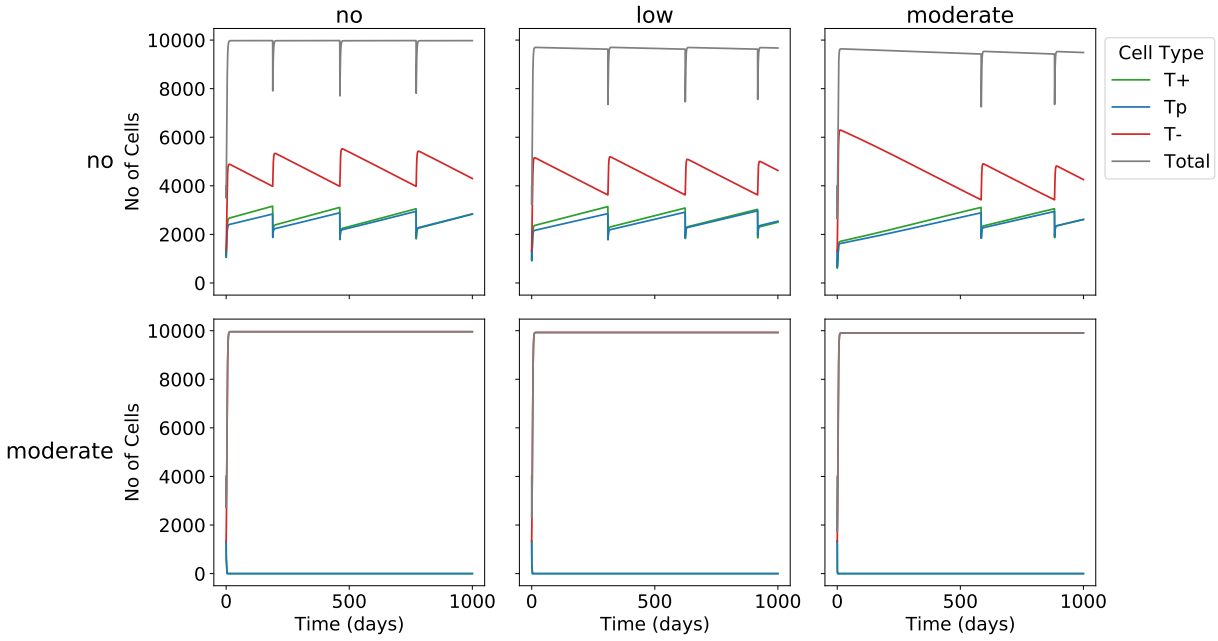


(c) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 2000

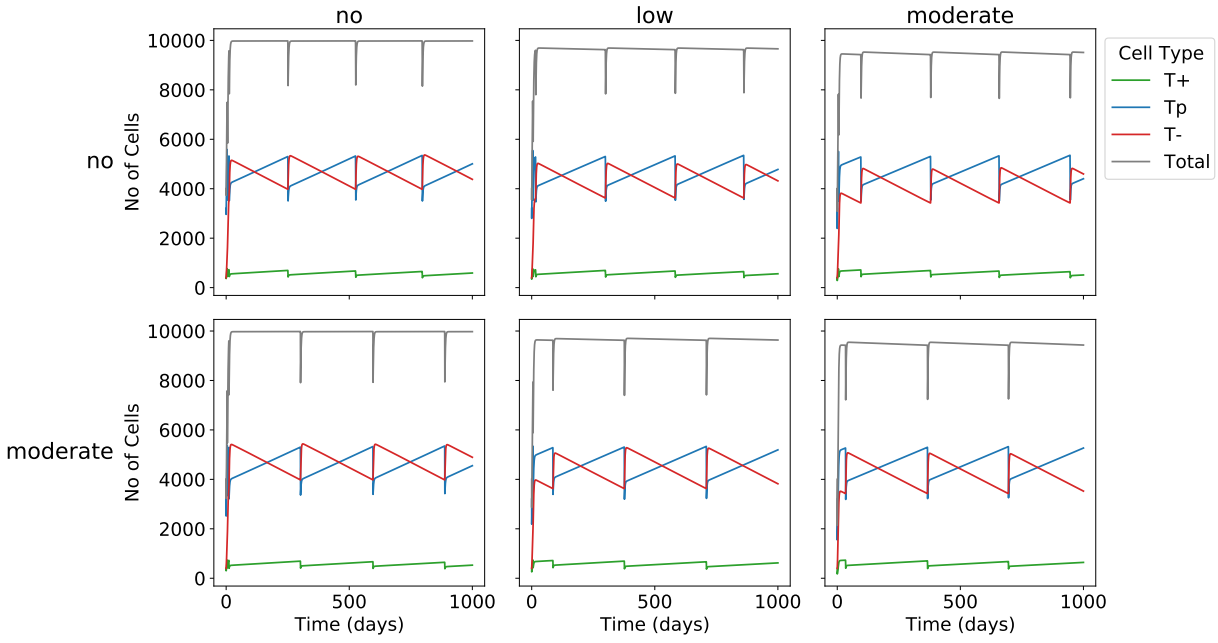


(d) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 2000

Figure 5.3: (Continued on next page)



(e) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 4000

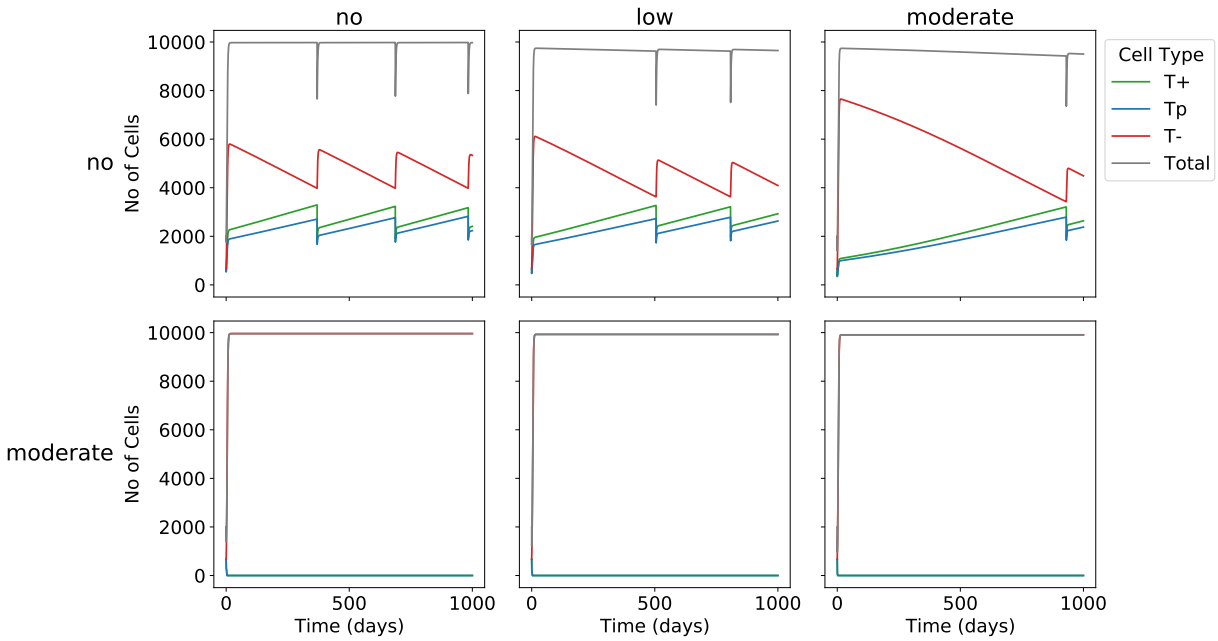


(f) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 4000

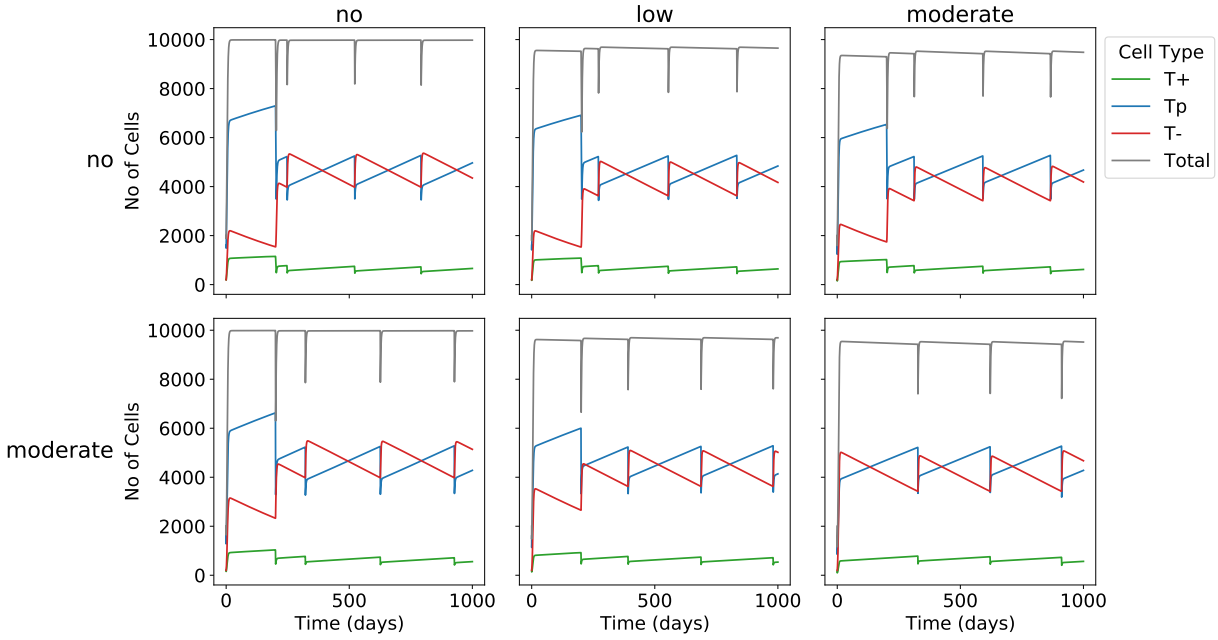
Figure 5.3: Time-series of all cell types with adaptive therapy (On : $6000, Off$: 4000) under different oxygen limitation (columns), testosterone limitation (rows) and initial seeding proportions, initial total seeding (subfigures).

5.2.2 With delay

As noted earlier, higher the amount of available testosterone, weaker the interspecific competition relative to intraspecific competition and therefore, better the coexistence. Since coexistence is tied strongly to the effectiveness of therapeutic outcomes, it stands to reason that the window of population sizes of T^p and T^+ used in adaptive therapy be chosen to allow for sufficient numbers of T^p and T^+ to remain in the population. Applying this idea specifically to the case with no limitation for either oxygen or testosterone, T^p and T^+ population fractions increase monotonously with time even without treatment. It is therefore possible that early treatment could be detrimental to coexistence by causing early competitive release of T^-, and it is tempting to speculate whether delaying the onset of treatment could improve therapeutic outcomes by allowing for a better balance of $T^p - T^+$ and T^-. However, the conditions that we have tested here, shown in Figure 5.4, do not show such an advantage to delay, possibly because we do not see much variability in the population fractions of each cell type within the delay time periods considered here. It is also important to note that this idea of delayed treatment does not account for the physiological cost of maintaining a growing tumour population until treatment is administered, which would be very important in a clinical setting.



(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 2000



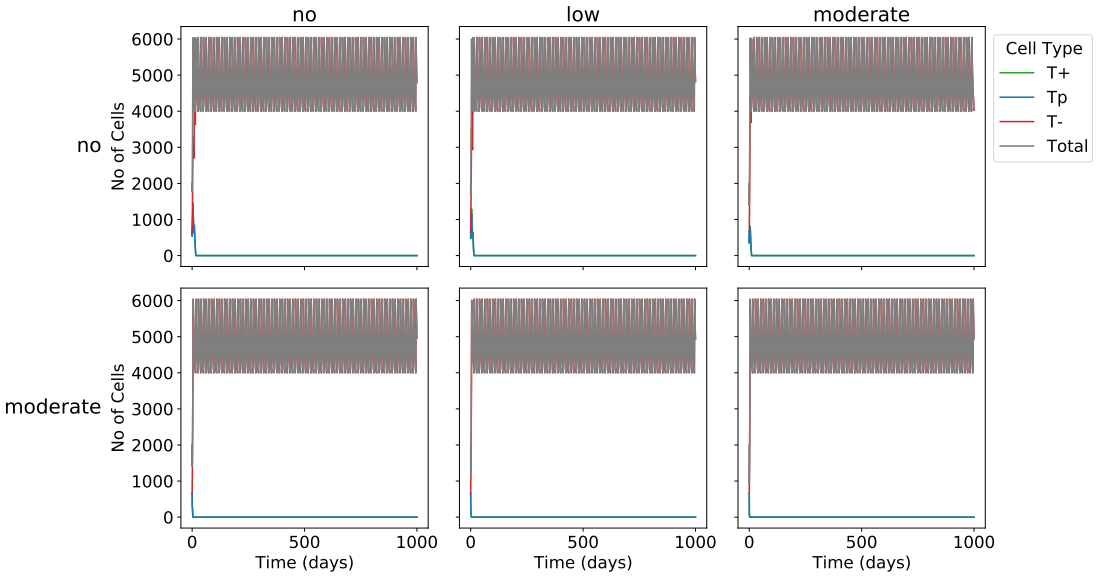
(b) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 2000

Figure 5.4: Time-series of all cell types with adaptive therapy (On : $6000, Off$: 4000) delayed by 200 days under different oxygen limitation (columns), testosterone limitation (rows) and initial seeding proportions (subfigures).

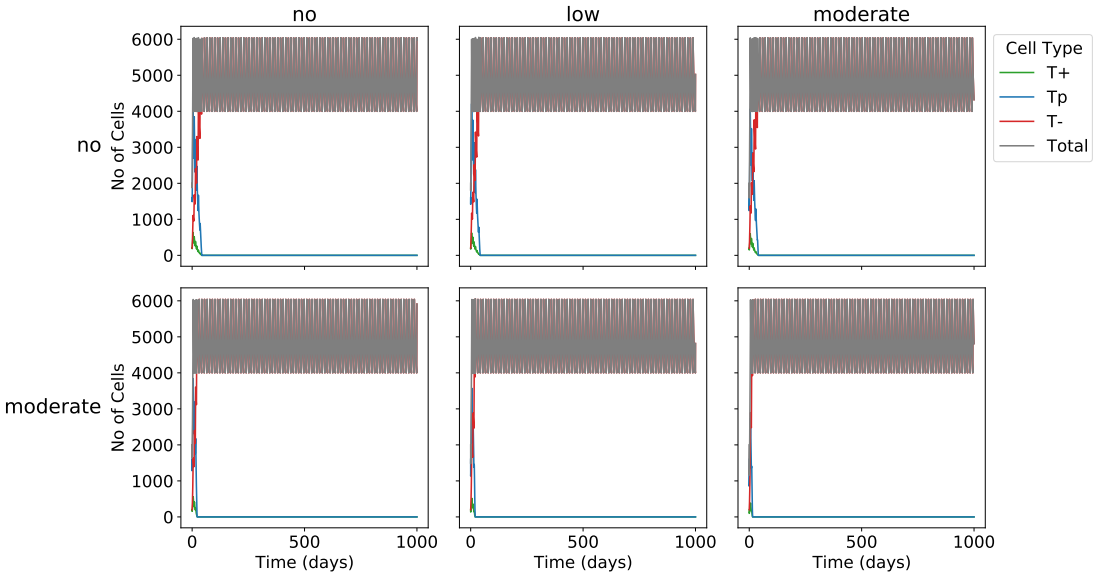
5.2.3 Combination therapy with docetaxel

Another aspect of prostate cancer therapy that could be of translational value to the model is combination therapy. Typically, hormone-specific agents like abiraterone are pairs with a general cytotoxic drug like docetaxel that causes cell death independent of cell type (West et al., 2019). A comprehensive exploration of combination therapy regimes would be time-consuming, but a smaller scale test-of-concept analysis was attempted here, in which AT was administered with both abiraterone and docetaxel; abiraterone was still responsive to $T^p - T^+$ alone, but docetaxel was administered based on thresholds of total population size.

Earlier data from AT with abiraterone alone leads to the expectation that there are potential spaces where docetaxel could be used to alleviate some of the T^- pressure on T^p and T^+, especially where they're driven to extinction. However, initial data visualised in Figure 5.5 show that the direct negative effect of docetaxel on T^p and T^+ outweigh any positive effect from reduction of T^- competition, suggesting that the scope for docetaxel application, at least based on the current modality, is highly limited in this system as it disrupts the sensitive balance of numbers between the three cell types. Nevertheless, more extensive testing is warranted.



(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 2000



(b) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 2000

Figure 5.5: Time-series of all cell types with combination adaptive therapy of abiraterone ($On : 6000, Off : 4000$; $T^+ + T^p$) and docetaxel ($On : 6000, Off : 4000$; $T^+ + T^p + T^-$) under different oxygen limitation (columns), testosterone limitation (rows) and initial seeding proportions (subfigures).

Chapter 6

Discussion

The findings of the study lead to the following points of general interest, beginning with the pairwise interactions. For the $T^p - T^-$ pairwise competition, the limitation of testosterone for T^p and that of oxygen for T^- has a major influence on competition and its outcomes. Increasing the limitation of a given resource for one cell relative to the other leads to the more-limited cell going extinct, regardless of specific identity. Only when the limitations are balanced between two types was coexistence observed. Resource levels can therefore act as control levers of the strength of competitive interactions between cell types and therefore determine the feasibility of coexistence. In addition to resource limitations, the relative initial seeding proportion of the cells can push the outcome in favour of the dominant cell. T^- in conventional terms has an advantage due to its shorter doubling time and the requirement of one less resource. A model that doesn't account for explicit resource dynamics and limitations might therefore predict that T^- always wins. Although competition coefficients can be made to disfavour T^- , justification for such an artefact isn't straightforward, particularly compared to the emergence of coexistence we observed in our model due to resource limitation effects.

Similar to $T^p - T^-$, the relative limitation for a given resource of one cell over the other, as well as the relative seeding proportion of the cells, both influence the outcomes of the $T^+ - T^p$ pairwise competition. Resource limitations can in fact be directly compared here unlike $T^p - T^-$, as both the cell types share the same qualitative resource dependencies. T^p has an advantage over T^+ with oxygen limitations as the latter requires the former for

testosterone. However, with testosterone limitation, T^p has a disadvantage relative to T^+ as it would be doubly growth-limited by both testosterone and T^+ density. Even though symmetric limitation of a resource across both the cell types produces a similar effect, the difference with testosterone is much more pronounced than with oxygen limitation, possibly indicating a stronger dependence on testosterone for these two cell types.

The general trend discussed above holds for the three-way competition as well. Due to the doubling time advantage of T^- and homogeneous limitations across cell types, testosterone limitations on T^p and T^+ have a higher influence on maintaining coexistence between the cells. In this context, it has been possible to identify zones of resource limitation for both testosterone and oxygen where the system goes from coexistence to T^- domination. Strong testosterone limitation without a numerical advantage of higher initial seeding density for T^p leads to T^- domination with no influence from oxygen. Weak testosterone limitation with a numerical advantage for T^p leads to coexistence with no influence of oxygen. Meanwhile, in the other cases where testosterone limitation is intermediate, the outcomes of competition are pushed either towards coexistence or T^- dominance by the oxygen limitation.

This framework used for studying competition alone then helps us understand the outcomes of therapy in mechanistic terms. T^p and T^+ are the only cell types to depend on testosterone and hence only they respond to abiraterone. SOC creates an additional limitation of testosterone by reducing the production rates and hence pushes these two cell types to extinction. AT would have no influence where T^p and T^+ are pushed to extinction by competition alone and maximum influence when the tumour is dominated by T^p and T^+. The resource limitations and seeding proportions therefore have an influence on the success of AT. The success of AT also depends crucially on the therapy window. Higher window would lead to better success, but at the increased physiological cost of maintaining a larger tumour. Meanwhile, with a smaller window achieving control would be more difficult and there would be a higher risk of competitive release. While this mechanistic information makes for a thorough understanding of the system from first principles, it also highlights the gap between the modelling approach and clinical reality. It is clear that application of such ecologically-aware treatment strategies would also require a quantum shift in the nature of information that can be realistically obtained from a cancer patient over meaningful timescales.

Insofar as eliminating the treatment-resistant cell type, all the therapy strategies we tried

have been a failure. But, some of this failure has been informative, and it has been possible to avoid or delay competitive release in some cases by maintaining a non-zero population of the responsive cell types T^+ and T^p. This is broadly in line with current thinking in the field regarding the goals of AT, which are more focused on achieving control than a complete cure. It is worth noting however, that the total tumour burden even when control was achieved was very close to the model's maximum effective carrying capacity. This could point to an important gap in the conceptualisation of AT that does not include the physiological cost of control over cure. It may then be worth investigating if AT could also be designed to address ways of minimising this cost alongside tumour control.

This study has been an attempt at a proof of concept to illustrate how ecological dynamics within a cancer system can inform progression as well as therapeutic decisions and outcomes. Based on its results so far, the following lines of further development are worth highlighting:

1. The exploration of combination therapy in this study has been limited. While the effects of docetaxel in the model were determined based on available experimental data, the ways in which it can be applied are still open modelling questions that can include the frequency of docetaxel, the magnitude of the dose, and the phase shift between abiraterone and docetaxel. Methods of combination therapy other than docetaxel are also known clinically (radiation, steroids, etc) and some of these can be incorporated into the framework of the current model to understand the dynamics of combination therapy better.

2. The cell types considered here are an oversimplification of actual cells in a biological system, which are highly variable in their functions and phenotypes. Cancer systems in particular can show an even higher degree of such heterogeneity due to their higher mutation rates and genomic instability. Bringing this to bear on the modelling approach we have taken, exploring a heterogeneity of cellular response across cell types to resource availability would be of interest here due to its strong influence on the outcomes of somatic competition and its effect on therapy. This is how pairwise competition has been explored in this study, and its extension to three-way competition should be informative.

3. In addition to heterogeneity, the cells can also switch their phenotype based on environmental conditions due to phenotypic plasticity. While some theoretical studies have explored the effect of mutational changes within the context of cell competition

(Snippert et al., 2014), phenotypic plasticity remains understudied. It is possible, however, that implementing plasticity or heterogeneous responses would be much easier with an individual based model, which opens up whole new possibilities of exploring the ecological dynamics of the system.

4. An individual-based model also allows for the addition of spatial heterogeneity to the system, which is an important component of biological variation in cancer populations. Solid tumours in particular have well-known gradients of resources between the tumour core and edge (Carmona-Fontaine et al., 2013), which could again open up even more channels of investigation.

These are possible ways in which the current study could be extended and broadened in scope. However, a more general comment on the modelling approach itself is also justified at this point.

Mathematical modelling is a really powerful tool in biology which helps in understanding a system without use of an actual system for the main experimentation. This can be very useful when experimenting on the actual system or biological model is not possible due to ethics, health risks, etc. However, one must keep in mind that all biological models are simplifications and involve many assumptions. Quoting (Box, 1979) "All models are wrong, but some are useful". There are no objective criteria for what makes a good model, or a fair set of prior assumptions. Every model makes assumptions that are manifestations of constraints inherent to the mathematical framework and data availability.

The concept of carrying capacity in a logistic or Lotka-Volterra system is a debated topic and even more so in cancer systems (Deisboeck & Wang, 2007; McLeod, 1997). Our model also assumes a carrying capacity derived from a rather arbitrary equilibrium value, y_i^*, given in Equation 2.10. However, this arbitrariness is partially alleviated since this carrying capacity is not explicitly fixed and instead is dynamically affected by the current concentration of resources. Nevertheless, it still suffers from the same setbacks of any model that invokes a carrying capacity, which is both difficult to define biologically (especially for cancer populations characterised by "uncontrolled" growth) and usually impossible to separate from the intrinsic growth rate. This leads to the possibility that a modelling approach that does not involve the carrying capacity at all where the resource availability is directly linked to growth rate in a consumer-resource type model may be better suited to study cancer systems that are typically at the edge of resource availability. Furthermore, comparing the differ-

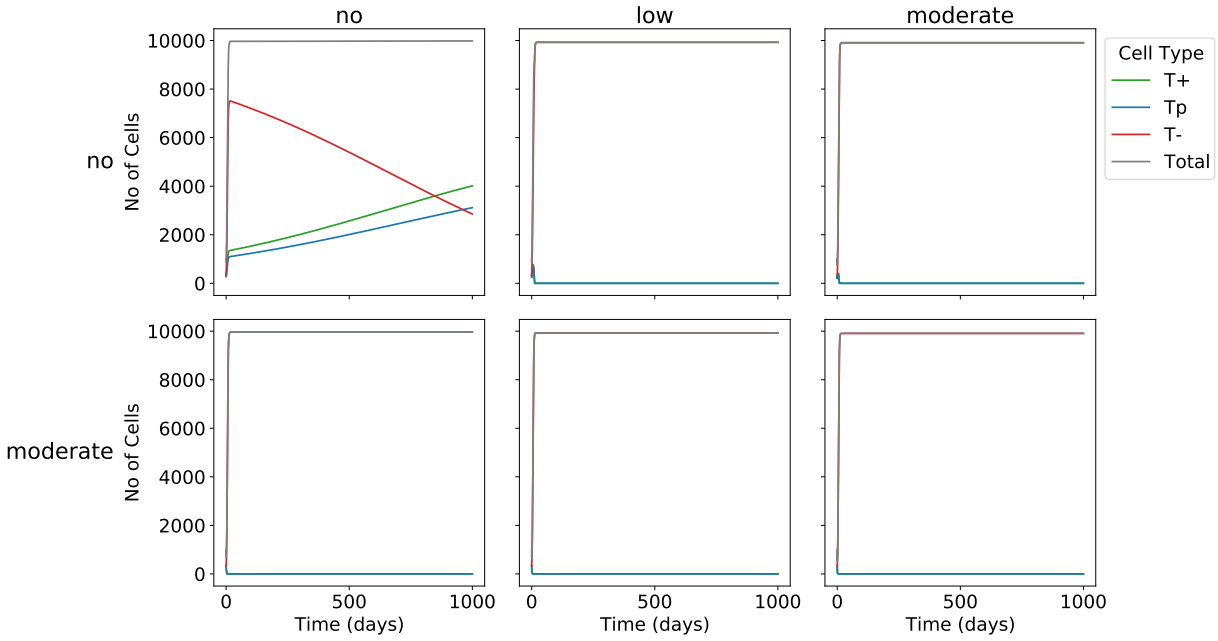
ences between such alternate modelling strategies could itself form an informative study of modelling assumptions and their impact on inferences.

The inherently exponential nature of the model made it very sensitive to parameter values and to small fluctuations in environmental conditions. This is borne out particularly clearly in how this system responds to the application of therapy almost instantaneously. The rapidity of these responses could likely hide subtler dynamics that may be more accessible to a different modelling approach that is designed to pick up on them.

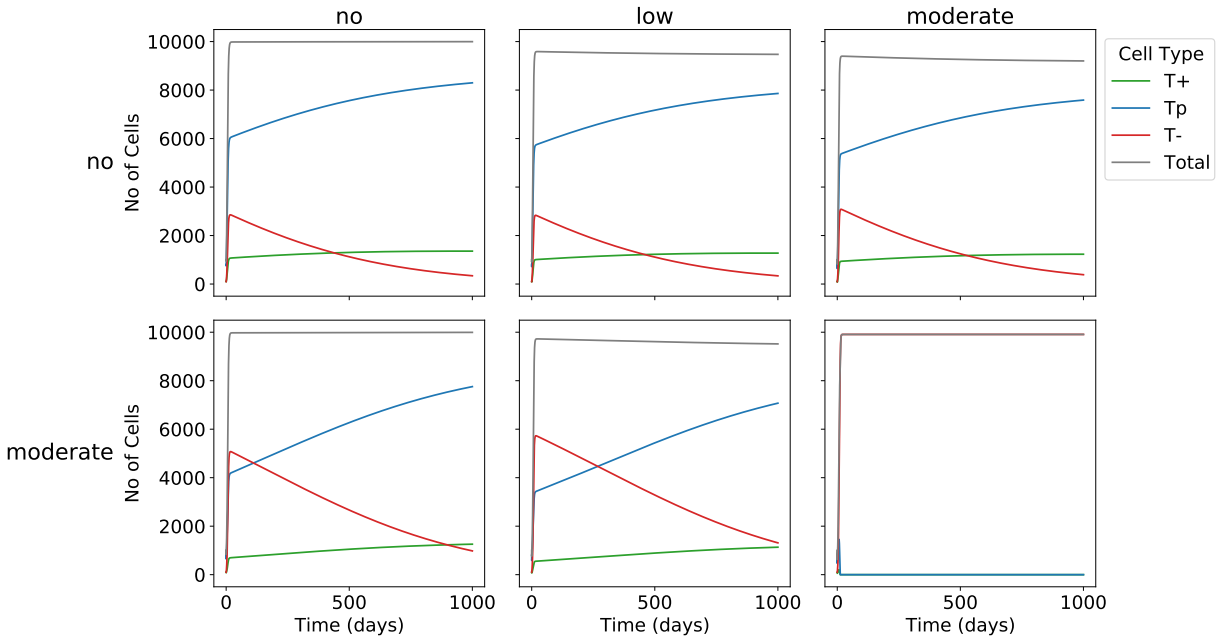
Our modelling approach has been more mechanistic in nature than data driven. We have started with parameters from fundamental processes governing the system for the overall behaviour to emerge out of it. A data driven model on the other hand, fits the parameters to clinical data. One could argue that such a data driven model reflects closer to reality since it follows the same dynamics, however, such models don't give valuable insight into these fundamental processes and act as black boxes. It may then be useful to explore ways of integrating clinical data more closely into mechanistic models, potentially resulting in mechanistic insight that can be applied more directly to clinical practice.

Appendix A

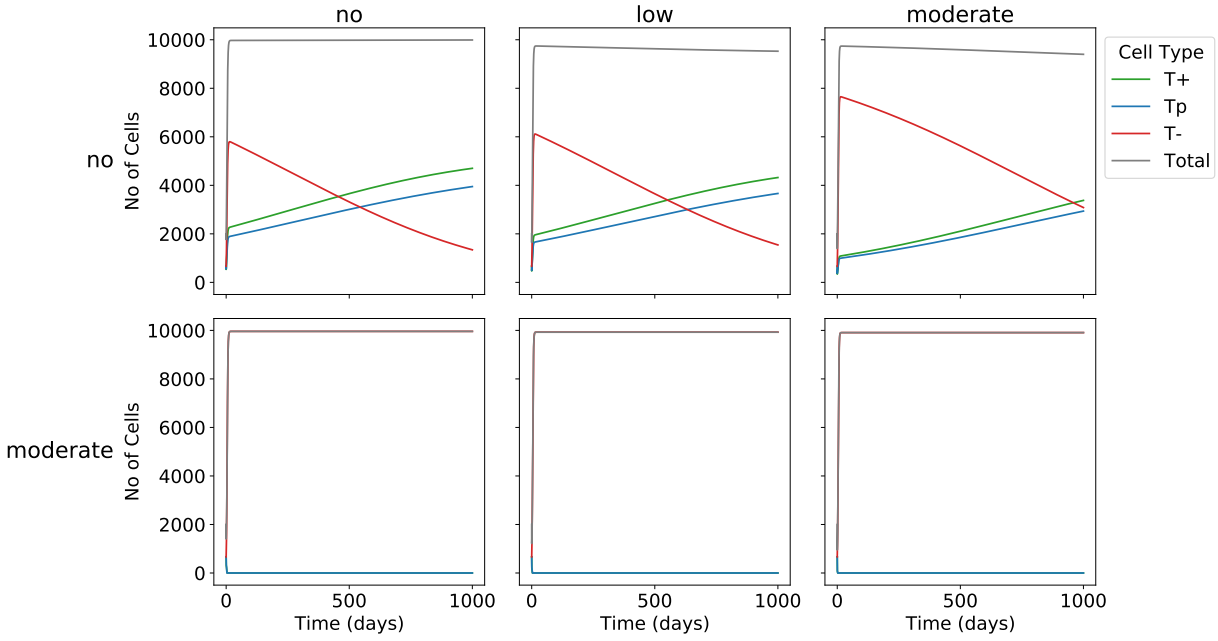
Supplementary



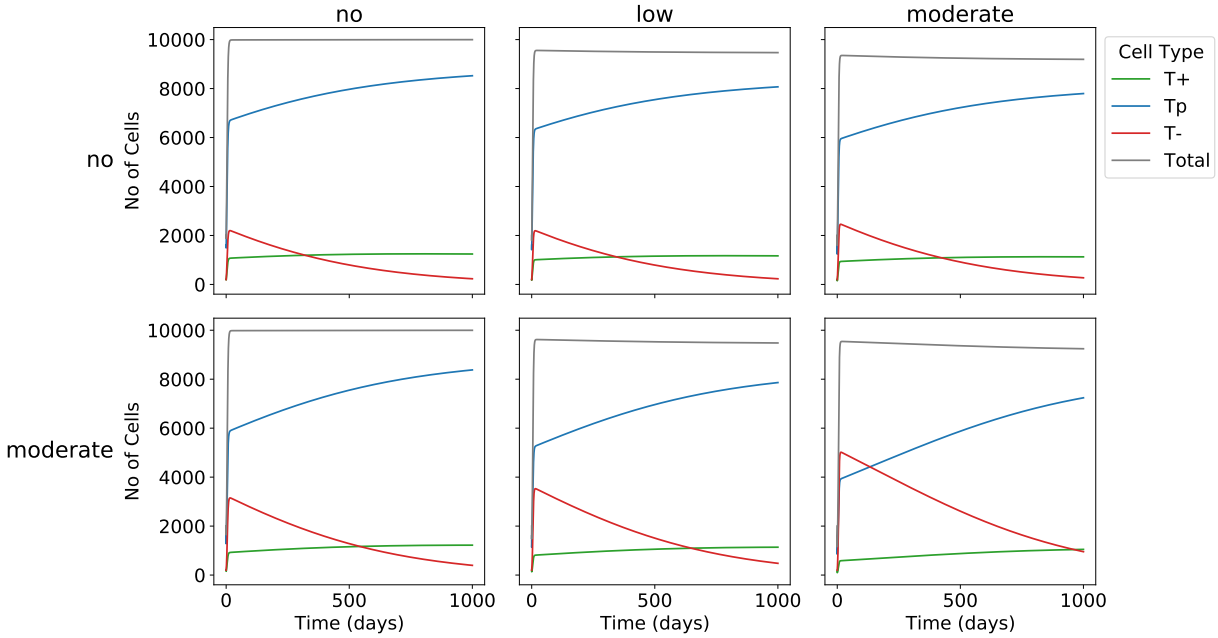
(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 1000



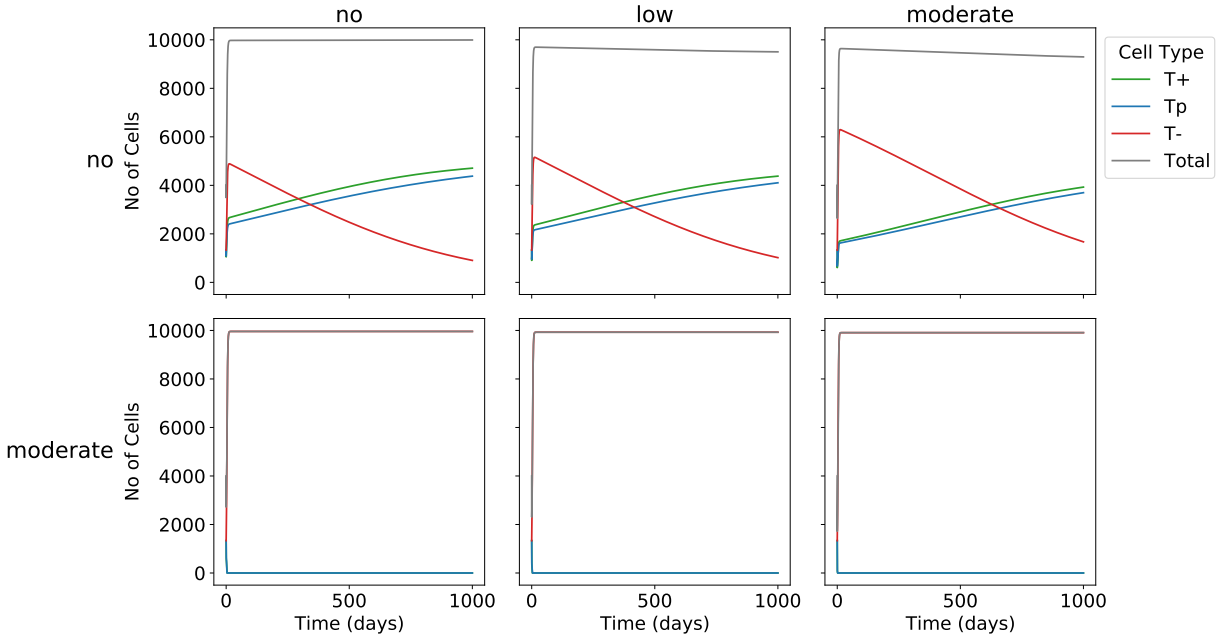
(b) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 1000



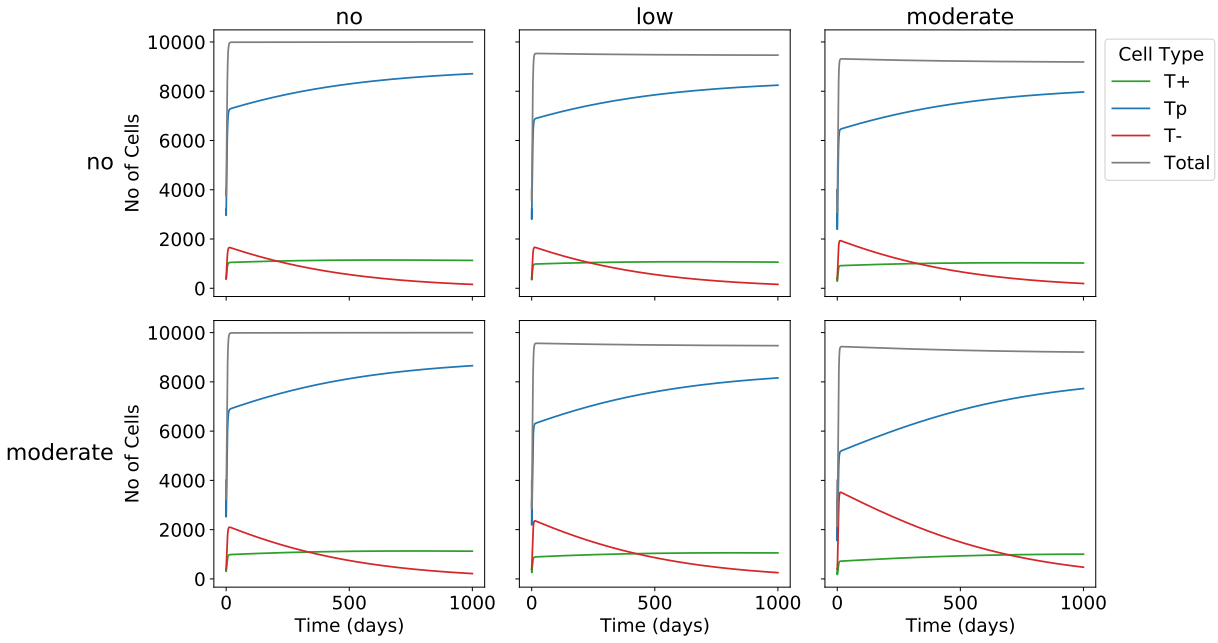
(c) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 2000



(d) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 2000

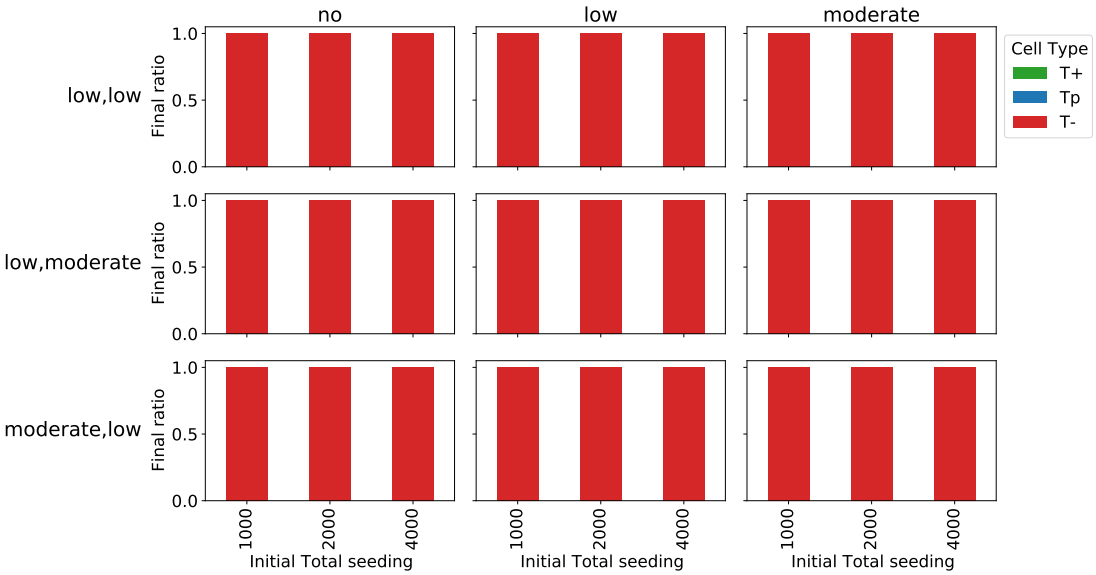


(e) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1, Initial Total seeding: 4000

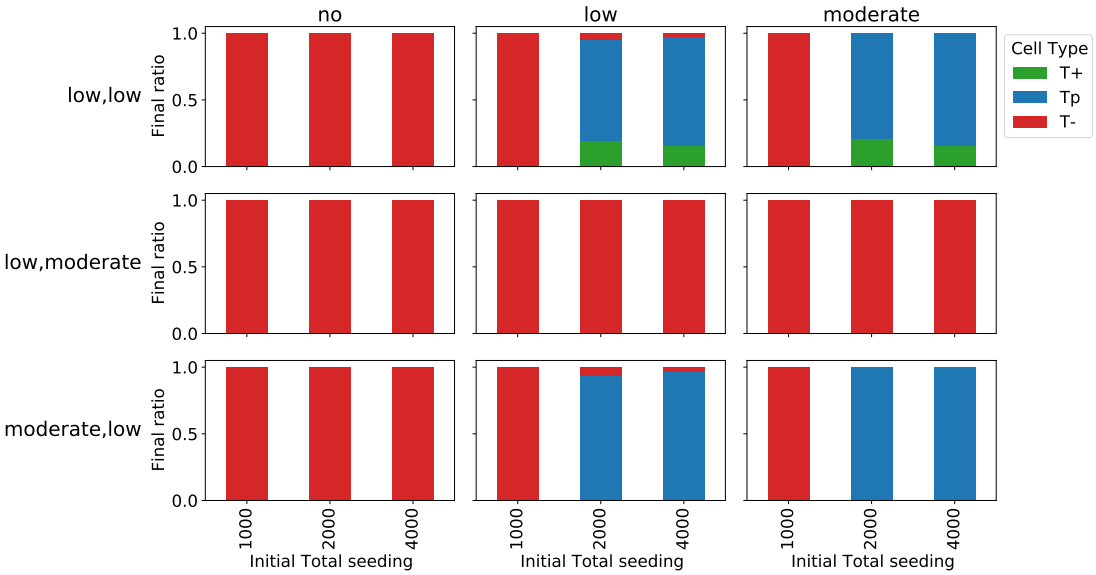


(f) High T^p seeding- $T^p : T^+ : T^-$ :: 8:1:1, Initial Total seeding: 4000

Figure A.1: Time-series of all 3 cell types under different oxygen limitation (columns), testosterone limitation (rows) and initial seeding proportions, initial total seeding (subfigures).



(a) Equal Seeding - $T^p : T^+ : T^-$ :: 1:1:1



(b) High T^p seeding- 8:1:1 :: $T^p : T^+ : T^-$

Figure A.2: Final ratio of all 3 cell types runs under different oxygen limitations of T^- (columns), oxygen efficiencies of T^+, T^p (rows) and initial seeding proportions (subfigures). T^p has low testosterone efficiency.

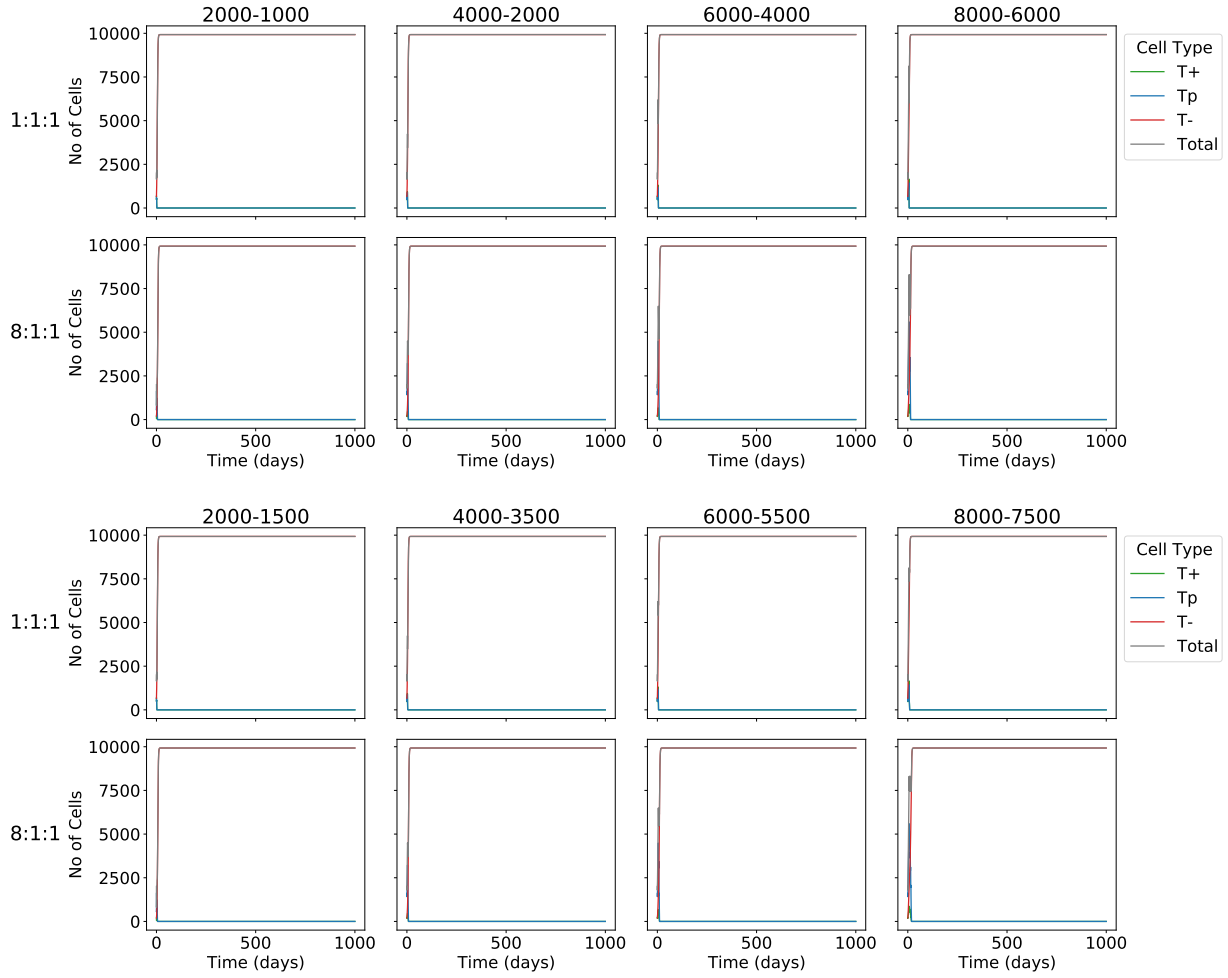


Figure A.3: Standardization of threshold considering all three cells for adaptive therapy, Columns: On-Off threshold, Rows: $T^p : T^+ : T^-$ Seeding

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