Studying effects of competition on adaptive therapy

A Thesis

submitted to
Indian Institute of Science Education and Research Pune
in partial fulfillment of the requirements for the
BS-MS Dual Degree Programme

by

Harshavardhan BV

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

July, 2021

Supervisor: Prof. Sutirth Dey

Certificate

This is to certify that this dissertation entitled Studying effects of competition on adaptive therapytowards 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 ?

Declaration

I hereby declare that the matter embodied in the report entitled Studying effects of competition on adaptive therapy 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

Acknowledgments

Not more than 250 words

Abstract

Write your abstract here

Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 What is Cancer?

Cancer is a collection of disease that is usually caused by uncontrolled division of cells and that has potential to spread to other parts of the body (NCI, 2021b). Cancer could be caused by various factors like tobacco usage, excess sun exposure, viral infection to name a few (Trichopoulos et al., 1996). Although, the underlying mechanism from these causes usually involves genetic mutations or epigenetic changes that alter the DNA. These alterations usually 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 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 mechanism of how cancer arises and then develop new therapies and drugs that target them. Although, the mortality among some types of cancer have been reduced significantly, we were not so lucky among other types of cancers and, the overall mortality still remains pretty high.

1.2 Conventional therapy against cancer

The most popular strategies to control cancer are radiotherapy, immunotherapy, surgery, and chemotherapy (NCI, 2021a). Radiotherapy involves using ionizing radiation to kill cancer cells. The high intensity radiation damages the DNA beyond repair and this causes the cells to stop dividing and die. Normal cells are also affected by the ionizing radiation and hence the radiation needs to be focussed to reduce collateral damage. Surgery involves removal of the tumour by cutting open the patient. The tumour may be removed in its entirety if it is localised but partial removal may be required to relieve patients of burden when complete removal would be life threatening. Immunotherapy involves triggering the immune system of the body to fight and kill the cancer cells. Immune system on its own can detect and kill abnormal cells but cancer cells can evolve mechanisms to evade these immune suppression. Immunotherapy supplements the immune system to better target and fight against these cells. Chemotherapy involves administering drugs, usually cytotoxic to kill cancer cells. Among chemotherapy, there are different variations, hormone therapy which suppresses hormones required by some cells to survive, targeted therapy which inhibits specific enzymes or antigens produced by cancer cells, and combination therapy which uses 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, Standard of Care (SOC) followed for most cancer is to administer the 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 fast as possible. This minimises the tumour burden quickly and should give better standard of living if it's the case.

However, evolutionarily thinking, a tumour would consist of cells with heterogeneous sensitivity towards a cytotoxic drug. Under normal conditions, that is, in the absence of therapy, these cells would compete with each other and keep the number of resistant phenotype in check. On administering the drug at MTD, the most sensitive cells are killed off first and this leads to a "competitive release" of the resistant phenotype (Scott & Marusyk, 2017). The resistant phenotype now grows without inhibition and takes over the population. These resistant phenotypes don't respond to further dose administered and the therapy fails. This is illustrated in Figure 1.1.

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.

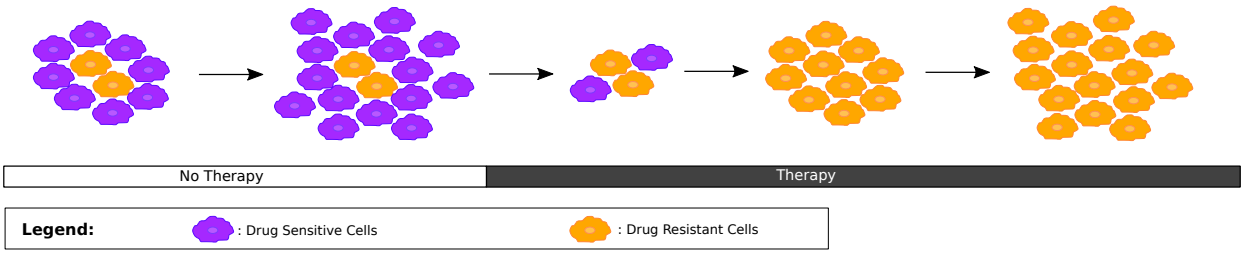


Figure 1.1: Illustration of competitive release under SOC

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. The best method would be to avoid such a competitive release in the first place. Research trend for cancer therapy has been towards development of new treatment protocols recently as a result.

Adaptive therapy (AT) is one such novel technique under development to avoid competitive release. In AT, the cytotoxic drug is administered at lower and fluctuating doses. This doesn't kill off all the sensitive cells and the probability of a competitive release is minimised. The resistant cells cannot take over due to competitive pressure from the still remaining sensitive cells and the tumour burden is maintained under control due to further doses being able to kill the sensitive cells that grow back. This is illustrated in Figure 1.2.

The dose administered at a given point is usually related to the tumour size at that given point (Gatenby et al., 2009). The challenge with designing AT regimens is to balance between the inhibition of resistant phenotype and the inhibition of the overall tumour size.

Even with this, AT may not be able to achieve control indefinitely. It'll only attempt to maximise the survival time compared to other regimens. AT, however, ignores the possibility of a cure, where the standard of care method would yield the best results. The patient has to live with the tumour for the rest of their life and other complications could arise due to this. (put in discussion maybe?)

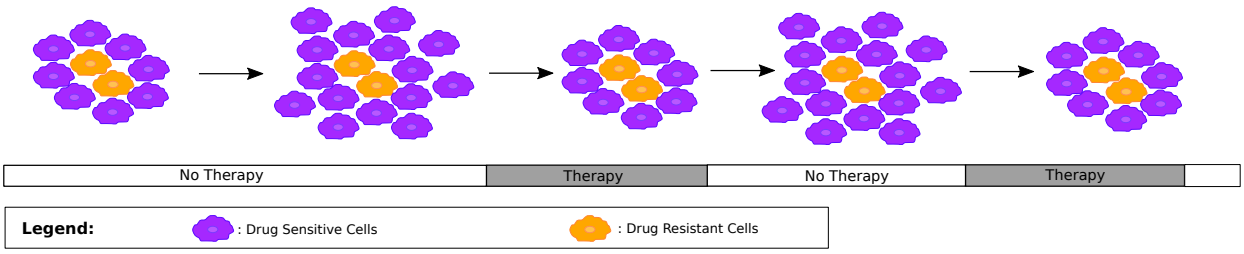


Figure 1.2: Illustration of control under AT

1.4 Importance of competition in adaptive therapy

The only way of controlling the resistant phenotype for a fixed drug in 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. Although, previously it was thought that resistant cells are required to have an inherent disadvantage for AT to be successful, even without it the survival time can be prolonged by competition between the cells (Strobl et al., 2020).

Cells can use different strategies such as higher proliferation rate, better survival at suboptimal conditions or lower death rate to compete with each other, and several such strategies are seen to be acquired over the course of cancer progression, as shown by the "hallmarks of cancer" framework (Hanahan & Weinberg, 2011).

1.5 System of Study

The metastatic castration resistant prostate cancer (mCRPC) was chosen to be the system of study. The mCRPC 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, 5α-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 mechanism is preserved and such prostate cancer remains testosterone dependent.

This system is usually modelled as consisting of three different types of cells: T^+, T^p and T^-. T^+ is the baseline population for prostate cancer which require testosterone for survival. 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. However, castration resistant prostate cancer soon develops, as the T^p cells can produce testosterone and sustain the T^+ cells. T^p cells are also dependent on testosterone, and they produce testosterone from cholesterol through upregulation of CYP17α (Dillard et al., 2008). The T^- cells on the other hand do not require testosterone as they have mutated ARs that remain active even in the absence of testosterone.

Abiraterone is a drug developed against mCRPC that inhibits the CYP17α and can be effective against both T^+ and T^p, however, not against T^-. And, this could lead to competitive release of the resistant T^- cells if administered in the standard clinical protocol. Abiraterone is usually administered after ADT as the system develops into a mCRPC. For our study, we shall only consider AT protocols on abiraterone under ADT.

1.6 Goal of the Project

The goal of the project is to:

1. Develop a model of the chosen system of study with their respective resource dependence.
2. Study the dynamics of the system under different conditions in the absence of therapy.
3. Compare the dynamics under effect of different therapy regimens.
4. Find the corresponding optimal therapy regimen that maximises the survival time for particular conditions.

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 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 $i \in \{T^+, T^p, T^-\}$

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

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 $res \in \{O_2, test\}$

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

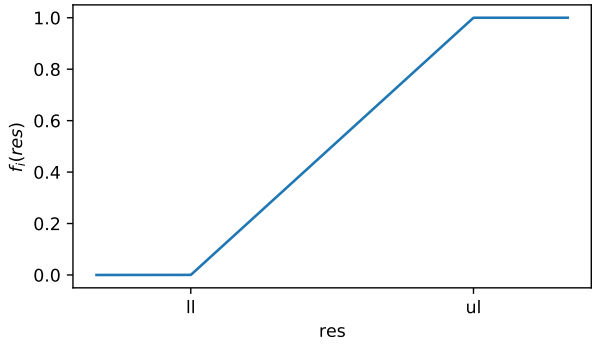


Figure 2.1: $f_i(res)$

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 oxygen 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.

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

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 Parameters Used

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 2.5 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.

$$r_i = \frac{ln(2)}{\tau_{d,i}} + \delta_i \tag{2.5}$$

Equation 2.6 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.

$$K_{i,max} = \frac{r_i}{r_i - \delta_i} y_i^* \tag{2.6}$$

Equation 2.7 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.

$$p_{O_2} = \lambda_{O_2} O_2^* + y_i^* \mu_i \tag{2.7}$$

Equation 2.8 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.

$$p_{test} - \mu_{test,T^p} = \frac{test^* \lambda_{test}}{y^*_{T^p}} = 4 \times 10^{-4} \tag{2.8}$$

2.3 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 run 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	Population growth rate of cell type i	T^+: 2.84×10^{-3} min^{-1} T^p: 2.79×10^{-3} min^{-1} T^-: 6.23×10^{-4} min^{-1}	Equation 2.5
δ_i	Population death rate of cell type i	T^+: 2.5×10^{-3} min^{-1} T^p: 2.5×10^{-3} min^{-1} T^-: 1.6×10^{-4} min^{-1}	(Jain et al., 2011)
$K_{i,max}$	Maximum Carrying capacity, coming up through the environment/resources	T^+: 8.35×10^4 T^p: 9.62×10^4 T^-: 1.34×10^4	Equation 2.6
$f_{i,res}$	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} $test$: 5×10^{-7} $\text{min}^{-1}\text{cell}^{-1}$	Equation 2.7, Equation 2.8
$\mu_{res,i}$	Uptake of resource res by cell type i	O_2, T^+: 1.63×10^{-6} $\text{min}^{-1}\text{cell}^{-1}$ O_2, T^p: 1.63×10^{-6} $\text{min}^{-1}\text{cell}^{-1}$ O_2, T^-: 1.04×10^{-6} $\text{min}^{-1}\text{cell}^{-1}$ $test$, T^+: 2.34×10^{-8} $\text{min}^{-1}\text{cell}^{-1}$ $test$, T^p: 6.00×10^{-8} $\text{min}^{-1}\text{cell}^{-1}$ $test$, T^-: 0 $\text{min}^{-1}\text{cell}^{-1}$	(Hail et al., 2010), Equation 2.8
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

Continued on next page

Parameter	Description	Value(s)	Source(s)
$ul_{res,i}$	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
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)

Table 2.1: Table of all parameters

Chapter 3

Results

3.1 Pairwise T^p - T^-

From the initial runs where two parameters were changed at a time, the following were observed:

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.

Additionally, a brute force parameter space exploration was done over a large combination of parameters. Due to the large parameter set, interpreting the results is difficult and only

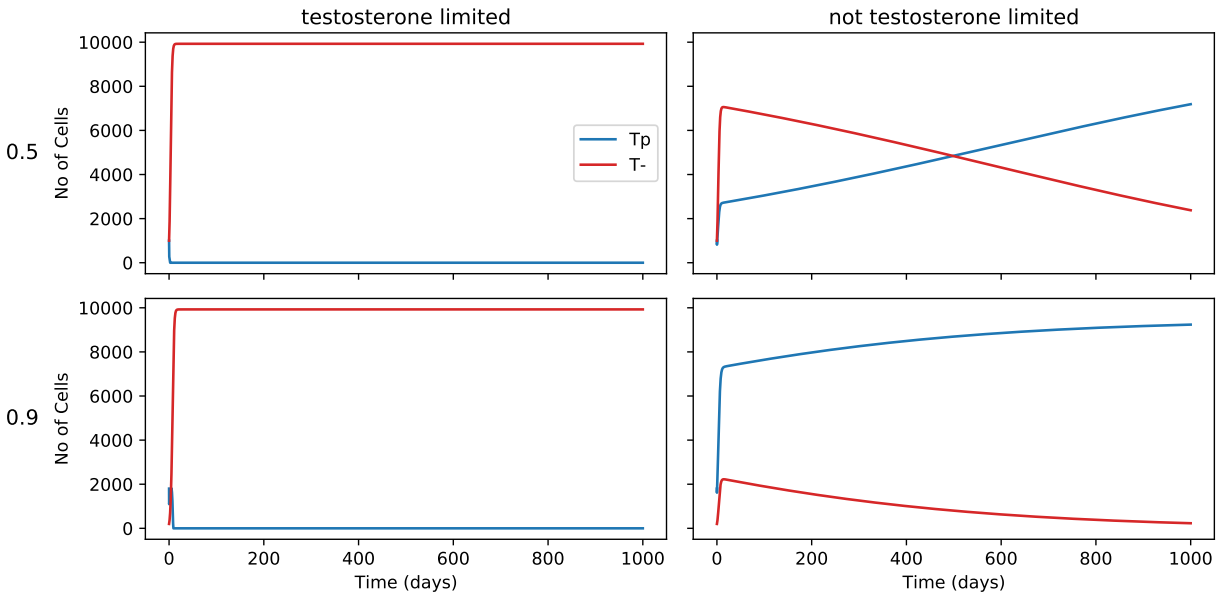


Figure 3.1: Pairwise $T^p - T^-$ timeseries, when T^p is testosterone limited and not testosterone limited (columns) and at different initial proportions of T^p (rows). T^p is testosterone limited at $ul_{test,T^p} = 0.5$ and not testosterone limited at $ul_{test,T^p} = 0.1$.

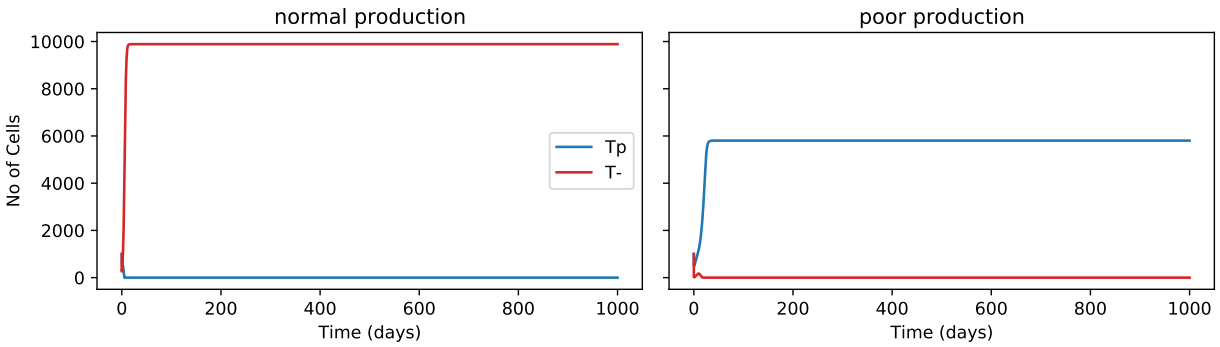


Figure 3.2: Pairwise $T^p - T^-$ timeseries, when T^- is oxygen limited and at different oxygen production (column). T^- is oxygen limited at $ll_{O_2,T^-} = 0.6$ and T^p is testosterone limited at $ul_{test,T^p} = 0.5$. The normal and poor production of oxygen are 0.11 and 0.0675 min^{-1} respectively

a few generalised observations were found, as 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 shouldn't be limited by oxygen 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.

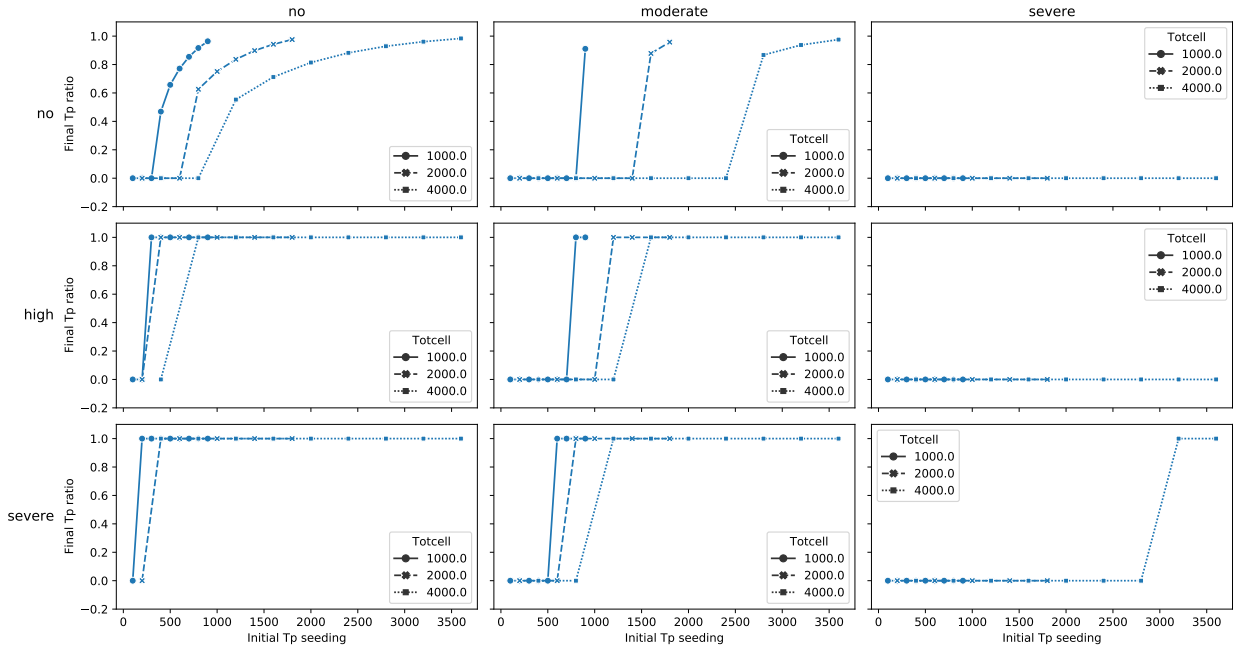
From the above observations, the following cases were formulated as an exhaustive formulation of possible conditions. Three levels each of T^p *test* limitation: no, moderate and severe corresponding to $ul_{test,T^p} = 0.1, 0.3, 1$ respectively, three levels each of T^- O_2 limitation: no, high and severe corresponding to $ll_{O_2,T^-} = 0, 0.6, 0.8$ respectively, and two levels each of O_2 production: normal and poor corresponding to $p_{O_2} = 0.11, 0.0675$ min^{-1} respectively were considered and pairwise competitive runs were done over all combinations of these with varying initial cell seeding.

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

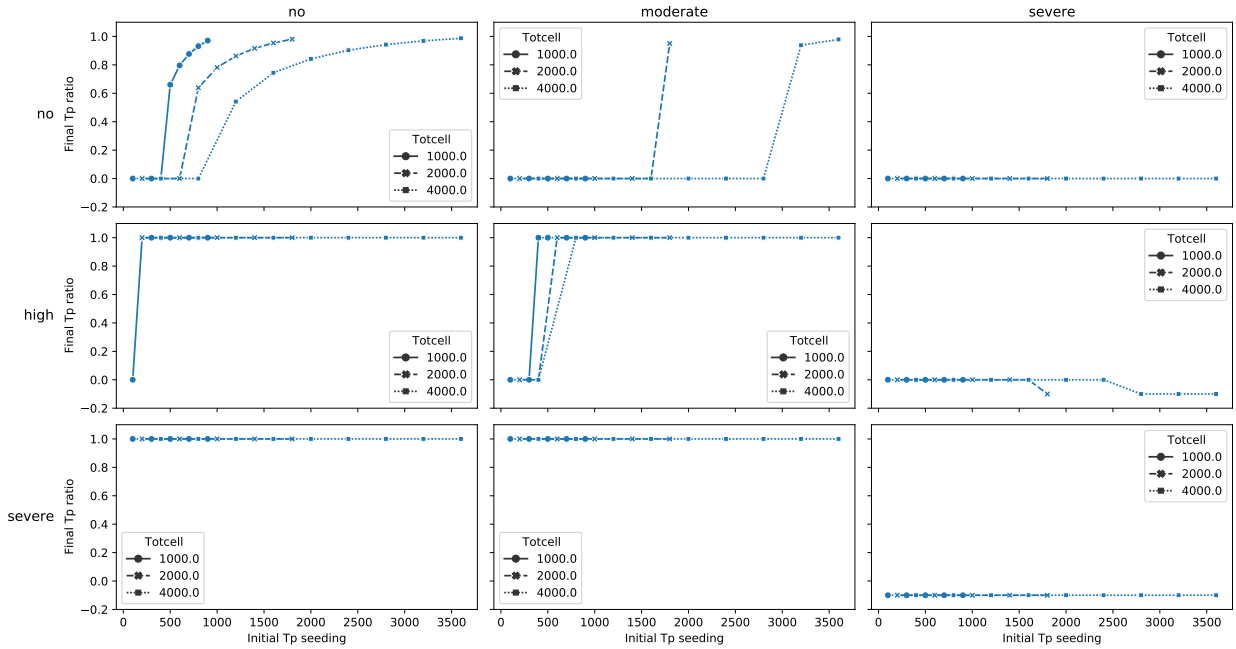
Continued on next page

O_2 **production**	T^- O_2 **limitation**	T^p *test* **limitation**
poor	severe	moderate
poor	severe	severe

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



(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.

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

1. Coexistence is observed only when there is low or moderate limitation of testosterone for T^p and no 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 co-existence, 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.

3.2 Pairwise T^+ - T^p

From the initial runs where two parameters were changed at a time, the following were observed:

1. When T^p limited by testosterone more than T^+ ($ul_{test,Tp} > ul_{test,T+}$), 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.

2. When T^p is weakly limited by testosterone relative to T^+ ($ul_{test,Tp} \leq ul_{test,T+}$), 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.

3. In the above case, the proportion of T^+ in the final population decreases as T^+ becomes more testosterone limited.

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.

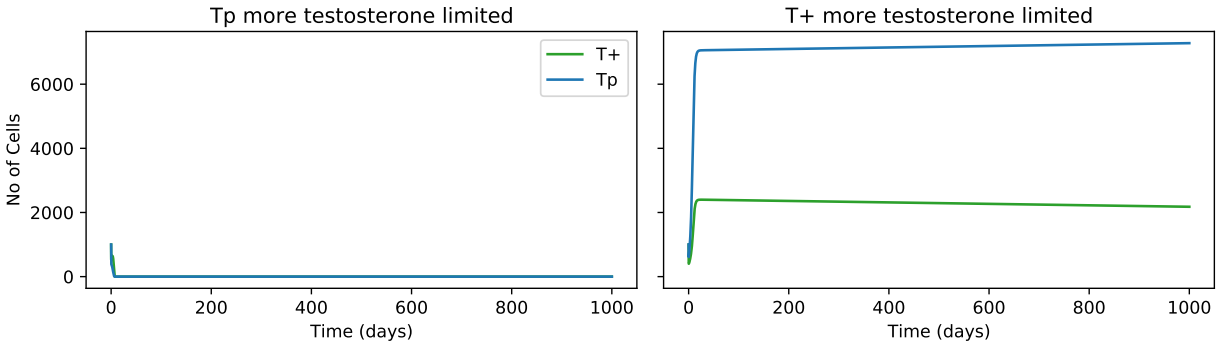


Figure 3.4: Pairwise $T^+ - T^p$ timeseries, when T^p is more testosterone limited than T^p and when T^+ is more testosterone limited than T^p. T^p is more limited testosterone limited at $ul_{test,T^+} = 0.3, ul_{test,T^p} = 0.5$ and T^+ is limited more at $ul_{test,T^+} = 0.5, ul_{test,T^p} = 0.3$.

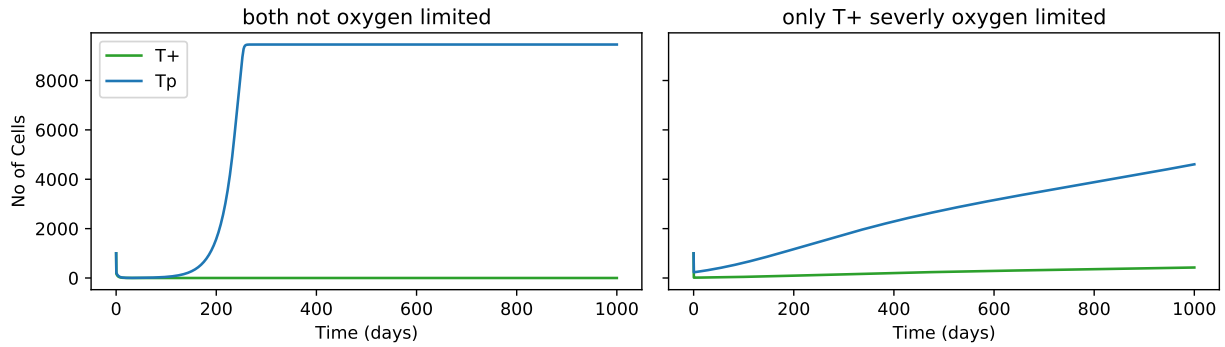


Figure 3.5: Pairwise $T^+ - T^p$ timeseries, when both cell types are testosterone limited and not oxygen limited at $ll_{O_2,T^+} = 0.0, ll_{O_2,T^p} = 0.0$ and T^+ is oxygen limited and T^p moderately at $ll_{O_2,T^+} = 0.6, ll_{O_2,T^p} = 0.4$.

From the above observations, the following cases were formulated as an exhaustive formulation of possible conditions. 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: no, 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 with varying initial cell seeding.

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 we're doing something roughly similar by adjusting the response function of the cell instead of actual resource concentrations.

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

Continued on next page

T^+ O_2 **limitation**	T^p O_2 **limitation**	T^+ *test* **limitation**	T^p *test* **limitation**
no	no	severe	moderate
no	no	severe	severe
no	moderate	no	no
no	moderate	no	moderate
no	moderate	moderate	no
no	moderate	moderate	moderate
no	severe	no	no
moderate	no	no	no
moderate	no	no	moderate
moderate	no	moderate	no
moderate	no	moderate	moderate
moderate	moderate	no	no
moderate	moderate	no	moderate
moderate	moderate	moderate	no
moderate	moderate	moderate	moderate
moderate	severe	no	no
severe	no	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 visualization, 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. Similarly, T^p is driven to extinction in every case where T^p limitation of either oxygen or testosterone is 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 co-existence.

3. However, with moderate limitation of oxygen for T^p relative to T^+. T^+ despite having a growth advantage due to the lowered oxygen limitation compared to T^p, still requires testosterone T^p for survival and reduces its growth and inhibition on T^p to allow it to secrete enough testosterone. Interestingly, this is also the case with co-existence at lower final T^p proportions than any other case.

4. In very broad terms, co-existence 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. However, under severe limitation of testosterone for both cell types, increasing relative proportion gives a competitive edge to T^p presumably by increasing net availability of testosterone in the system. This increased availability also has limits beyond which further increase in T^p proportion is marginally detrimental to T^p success.

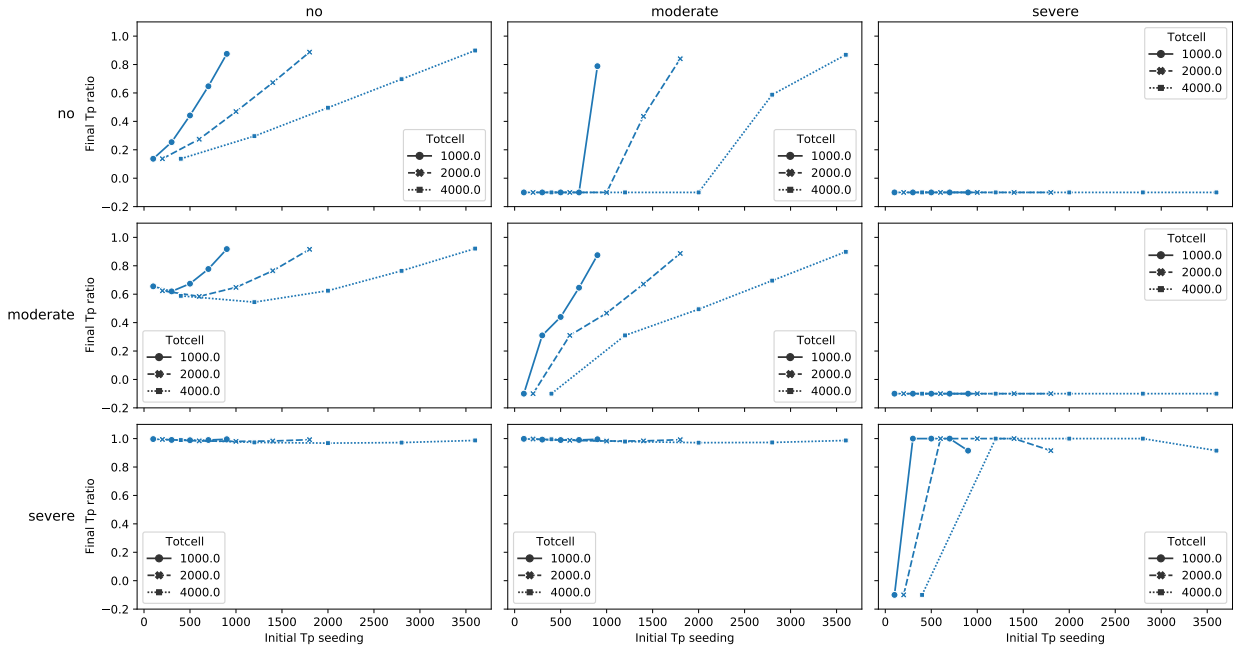
5. Similarly, co-existence is observed when T^+ is moderately testosterone limited relative to T^p. However, in this case, a lower initial proportion of T^p favours T^p and leads to a dip in the plot. At a low initial proportion of T^p, T^+ being limited by testosterone dies out until sufficient testosterone 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 when both the cells are moderately limited either by testosterone or oxygen is very similar to the cases when both the cells were not limited by either testosterone or oxygen respectively. 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 testosterone and a higher final T^p population compared to the case without any testosterone limitations.

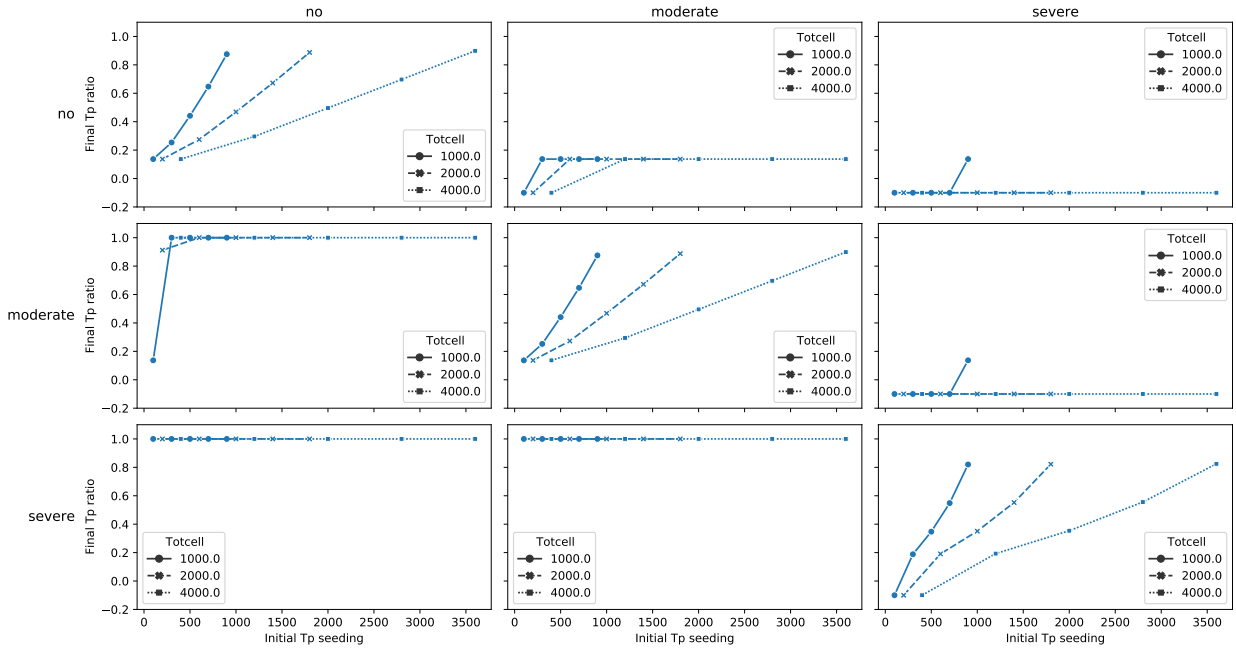
7. When both testosterone and oxygen are moderately limiting for a cell type relative to the other, the combined overall limitation is severe and that particular cell type is driven to extinction similar to when only one resource was severely limiting.

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 going extinct and T^+ in extension at low initial proportion to T^+ going extinct for higher initial proportion.

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

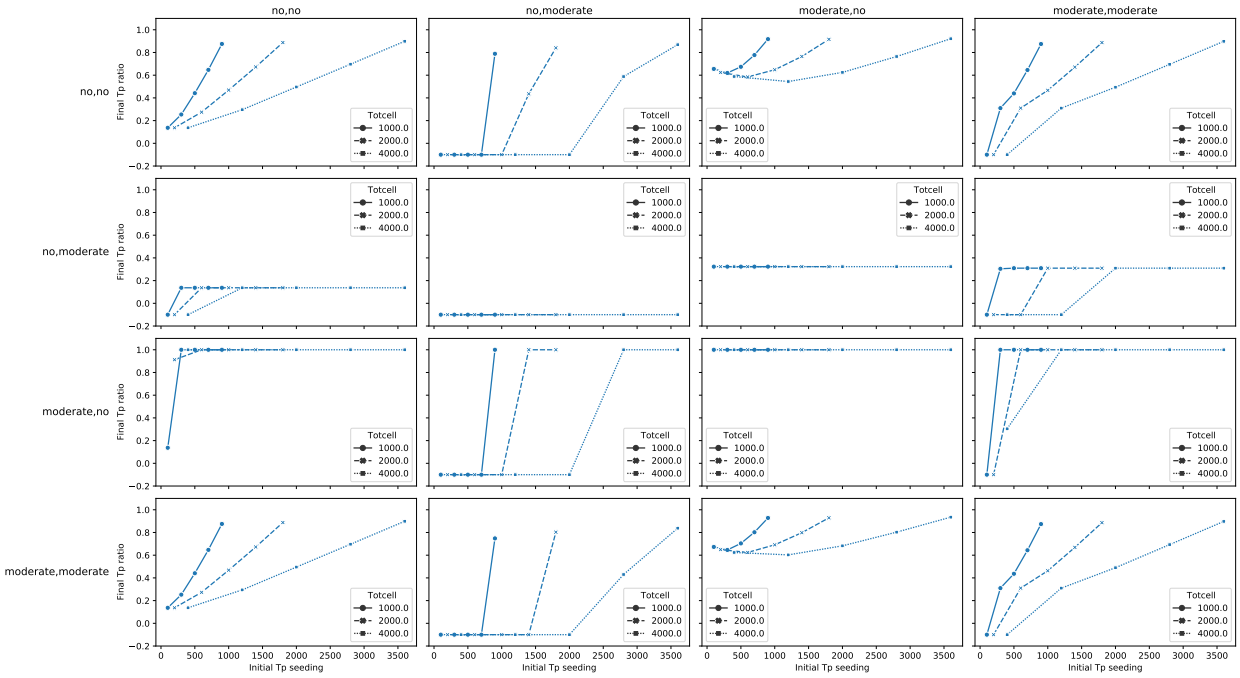


(a) *test* limitations. Columns: T^p *test* limitation, Rows: T^+ *test* limitation.



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

Figure 3.6: 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.



(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. (cont.)

3.3 All 3 celltypes

3.3.1 Efficiency

To simplify things, the resource limitations as described in the previous sections can instead be considered as efficiencies of the cell types in utilization of a particular resource. A cell with a high efficiency for a resource would be limited less by that resource whereas a cell with low efficiency for a resource would be severely limited by that resource.

With all three cell types, the number of combinations and permutations increase combinatorially, and sifting through such a massive pile of data is a daunting prospect. So, we are starting with a simpler case of the same strategy for all three cell types. The different efficiency levels and their corresponding values for lower and upper limits are given in Table 3.3.

Resource	Efficiency	lower limit	upper limit
Oxygen	Low Efficiency	0.4	1.0
	Null Efficiency	0.0	1.0
	High Efficiency	0.0	0.1
Testosterone	Low Efficiency	0	0.3
	High Efficiency	0	0.1

Table 3.3: Table of limits corresponding to efficiencies for different resources

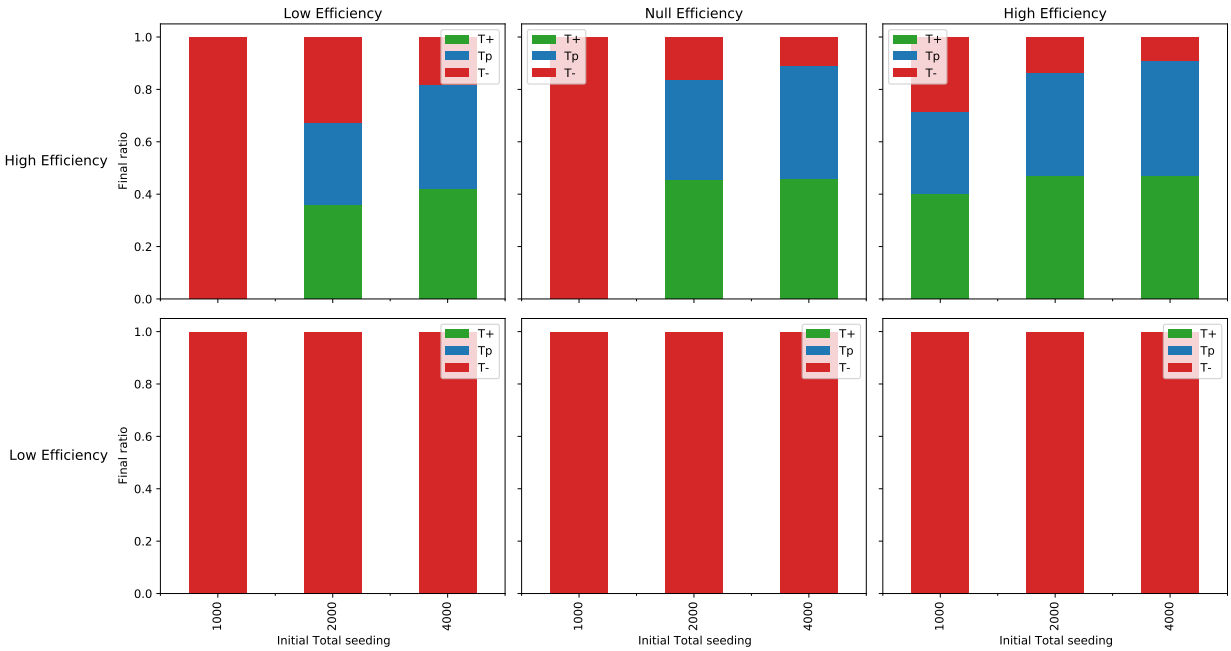
The following were observed from the different cases as visualised is Figure 3.7.

1. Barring the case with high efficiencies for both testosterone and oxygen, T^p and T^+ goes extinct for the lowest equal seeding densities with the total amounting to 1000.

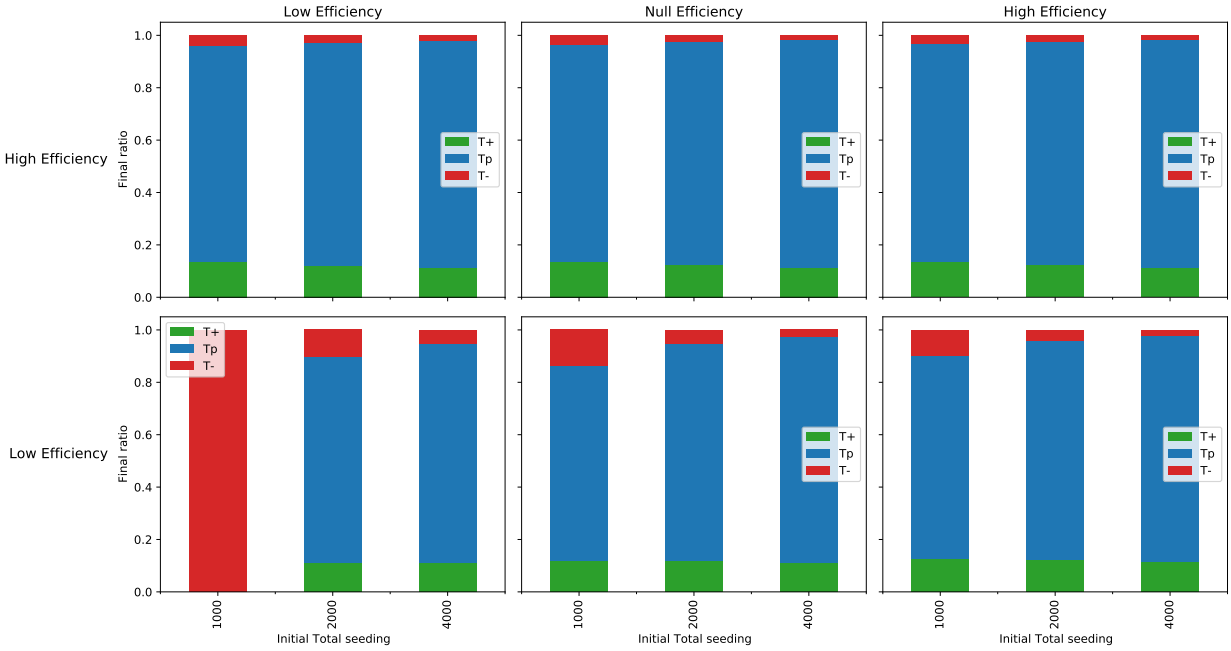
2. With low testosterone efficiency, T^p and T^+ go extinct at all the equal seeding densities regardless of their oxygen efficiencies. T^- isn't affected by the testosterone limitation and can outcompete the other cells. However, when T^p is seeded at 8 times the density of the other cell types, co-existence is achieved other than with low oxygen efficiency.

3. T^- seems to have an advantage when the oxygen efficiencies are lower. While this difference can be seen clearly with the reduction in final T^- ratio from low to null oxygen efficiency, the difference between null and high oxygen efficiency is minor.

4. Higher initial total seeding favours T^p and T^+ as the higher T^p numbers lead to more testosterone secreted in the environment, and T^p and T^+ can outcompete T^-. However, when T^p is seeded at 8 times the density of the other cell types with high testosterone efficiency, they have reached their saturation levels and the difference between the total seedings are minor.



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



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

Figure 3.7: Final ratio of all 3 cell types runs under different oxygen efficiency (columns), testosterone efficiency (rows) and initial seeding proportions (subfigures).

Bibliography

ATCC. (2021). Atcc: The global bioresource center. https://www.atcc.org/

Cunningham, J. J., Brown, J. S., Gatenby, R. A., & Staňková, K. (2018). Optimal control to develop therapeutic strategies for metastatic castrate resistant prostate cancer. *Journal of Theoretical Biology*, *459*, 67–78. https://doi.org/https://doi.org/10.1016/j.jtbi.2018.09.022

Dillard, P. R., Lin, M.-F., & Khan, S. A. (2008). Androgen-independent prostate cancer cells acquire the complete steroidogenic potential of synthesizing testosterone from cholesterol. *Molecular and Cellular Endocrinology*, *295*(1), 115–120. https://doi.org/https://doi.org/10.1016/j.mce.2008.08.013

Frei, E., & Canellos, G. P. (1980). Dose: A critical factor in cancer chemotherapy. *The American Journal of Medicine*, *69*(4), 585–594. https://doi.org/https://doi.org/10.1016/0002-9343(80)90472-6

Gatenby, R. A., Silva, A. S., Gillies, R. J., & Frieden, B. R. (2009). Adaptive therapy. *Cancer Research*, *69*(11), 4894–4903. https://doi.org/10.1158/0008-5472.CAN-08-3658

GRØNBÆK, K., HOTHER, C., & JONES, P. A. (2007). Epigenetic changes in cancer. *APMIS*, *115*(10), 1039–1059. https://doi.org/https://doi.org/10.1111/j.1600-0463.2007.apm_636.xml.x

Hail, N., Chen, P., & Bushman, L. R. (2010). Teriflunomide (leflunomide) promotes cytostatic, antioxidant, and apoptotic effects in transformed prostate epithelial cells: Evidence supporting a role for teriflunomide in prostate cancer chemoprevention. *Neoplasia*, *12*(6), 464–475. https://doi.org/https://doi.org/10.1593/neo.10168

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *cell*, *144*(5), 646–674.

Heinlein, C. A., & Chang, C. (2004). Androgen Receptor in Prostate Cancer. *Endocrine Reviews*, *25*(2), 276–308. https://doi.org/10.1210/er.2002-0032

Jain, H. V., Clinton, S. K., Bhinder, A., & Friedman, A. (2011). Mathematical modeling of prostate cancer progression in response to androgen ablation therapy. *Proceedings of the National Academy of Sciences*, *108*(49), 19701–19706. https://doi.org/10.1073/pnas.1115750108

Moolgavkar, S. H., & Knudson, A. G. (1981). Mutation and Cancer: A Model for Human Carcinogenesis2. *JNCI: Journal of the National Cancer Institute*, *66*(6), 1037–1052. https://doi.org/10.1093/jnci/66.6.1037

NCI. (2021a). Types of cancer treatment. https://www.cancer.gov/about-cancer//treatment/types

NCI. (2021b). What is cancer? https://www.cancer.gov/about-cancer/understanding/what-is-cancer

Scott, J., & Marusyk, A. (2017). Somatic clonal evolution: A selection-centric perspective [Evolutionary principles - heterogeneity in cancer?]. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1867*(2), 139–150. https://doi.org/https://doi.org/10.1016/j.bbcan.2017.01.006

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer statistics, 2021. *CA: A Cancer Journal for Clinicians*, *71*(1), 7–33. https://doi.org/https://doi.org/10.3322/caac.21654

Stewart, G. D., Ross, J. A., McLaren, D. B., Parker, C. C., Habib, F. K., & Riddick, A. C. (2010). The relevance of a hypoxic tumour microenvironment in prostate cancer. *BJU International*, *105*(1), 8–13. https://doi.org/https://doi.org/10.1111/j.1464-410X.2009.08921.x

Strobl, M., West, J., Viossat, Y., Damaghi, M., Robertson-Tessi, M., Brown, J., Gatenby, R., Maini, P., & Anderson, A. (2020). Turnover modulates the need for a cost of resistance in adaptive therapy. *bioRxiv*. https://doi.org/10.1101/2020.01.22.914366

Titus, M. A., Schell, M. J., Lih, F. B., Tomer, K. B., & Mohler, J. L. (2005). Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clinical Cancer Research*, *11*(13), 4653–4657. https://doi.org/10.1158/1078-0432.CCR-05-0525

Trichopoulos, D., Li, F. P., & Hunter, D. J. (1996). What causes cancer? *Scientific American*, *275*(3), 80–87. http://www.jstor.org/stable/24993351

Zhang, J., Cunningham, J. J., Brown, J. S., & Gatenby, R. A. (2017). Integrating evolutionary dynamics into treatment of metastatic castrate-resistant prostate cancer. *Nature Communications*, *8*(1), 1816. https://doi.org/10.1038/s41467-017-01968-5