Studying effects of competition on adaptive therapy

A Thesis

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by

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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

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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 (cancer.gov, 2015). 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, chemotherapy, immunotherapy, and surgery. 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 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.

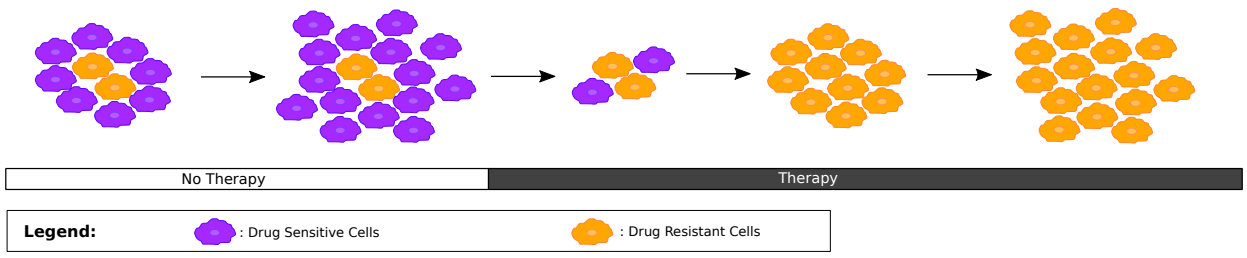


Figure 1.1: Illustration of competitive release under SOC

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.

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?)

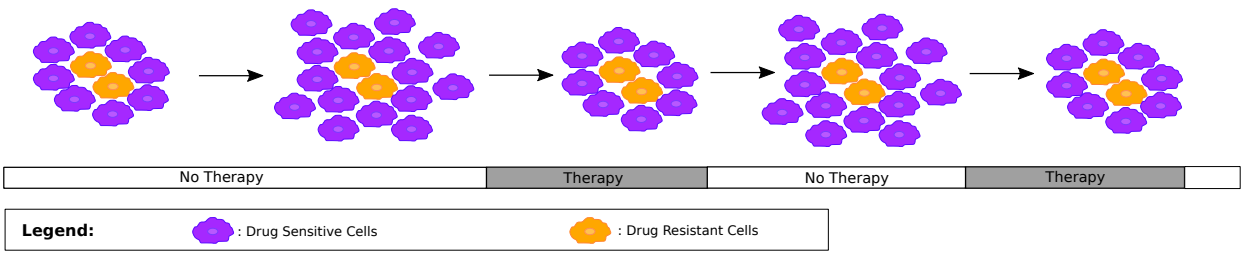


Figure 1.2: Illustration of control under AT

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

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

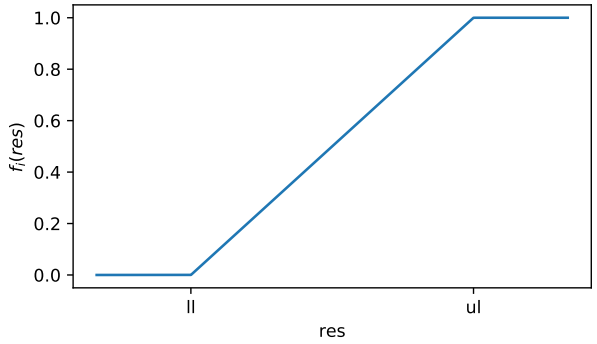


Figure 2.1: $f_i(res)$

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.org, 2020)
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

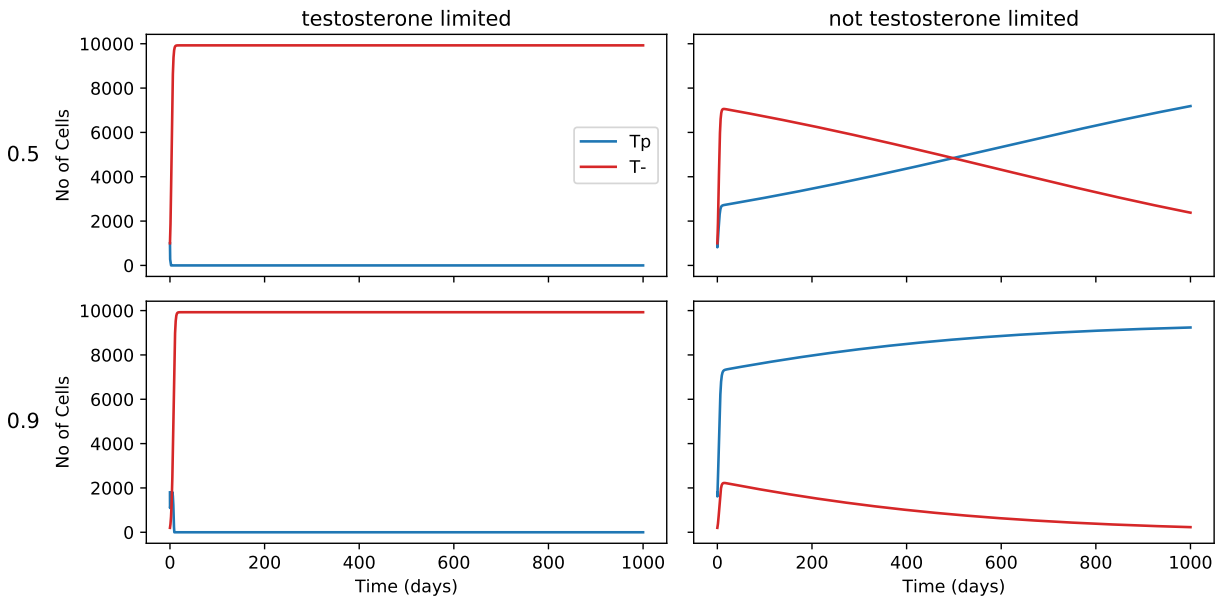


Figure 3.1: Pairwise $T^p - T^-$ timeseries, when T^p is testosterone limited and not testosterone limited (columns) and at different initial proportions of T^p (rows). T^p is testosterone limited at $ul_{test,T^p} = 0.5$ and not testosterone limited at $ul_{test,T^p} = 0.1$.

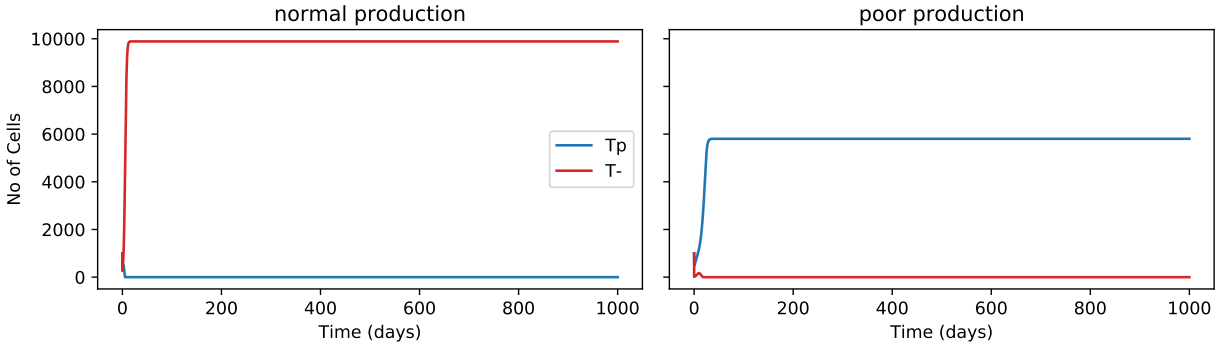


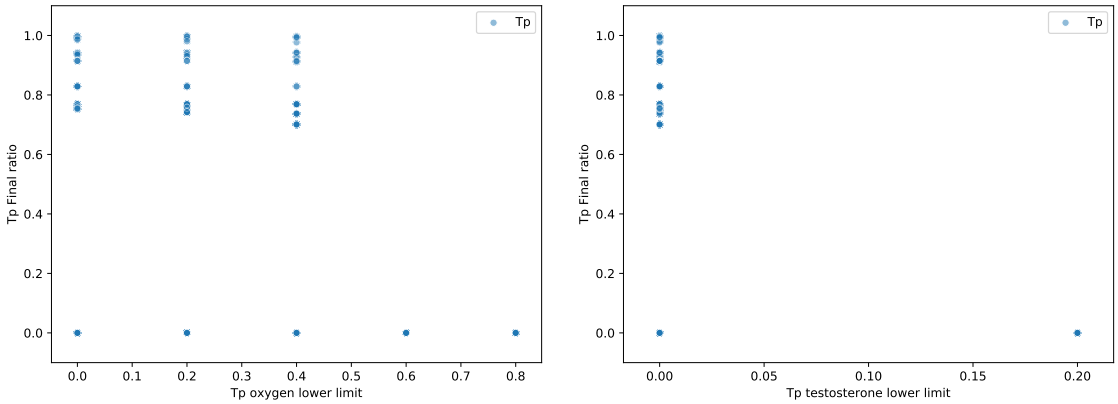
Figure 3.2: Pairwise $T^p - T^-$ timeseries, when T^- is oxygen limited and at different oxygen production (column). T^- is oxygen limited at $ll_{O_2,T^-} = 0.6$ and T^p is testosterone limited at $ul_{test,T^p} = 0.5$. The normal and poor production of oxygen are 0.11 and 0.0675 min^{-1} respectively

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^-. This is visualised in Figure 3.3a.

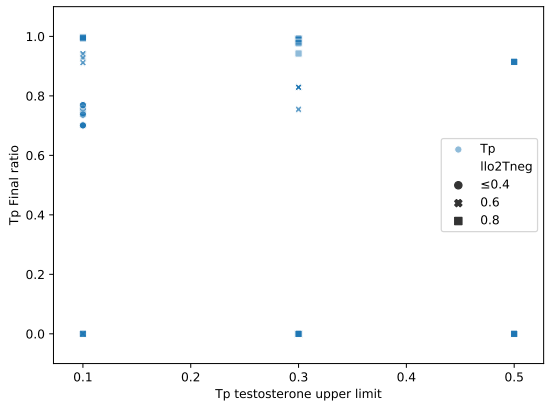
2. T^- drives T^p to extinction when $ll_{test,T^p} \geq 0.2$, regardless of the other parameters, in other words, T^p needs to be able to grow even on the smallest amount of testosterone to compete with T^-. This is visualised in Figure 3.3b.

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. This is visualised in Figure 3.3c.



(a) Lower limit of oxygen for T^p

(b) Lower limit of testosterone for T^p



(c) Upper limit of testosterone for T^p

Figure 3.3: Final T^p ratio of pairwise $T^p - T^-$ runs vs parameters. Note: The multiple points for same x-value represents values on varying the other parameters.

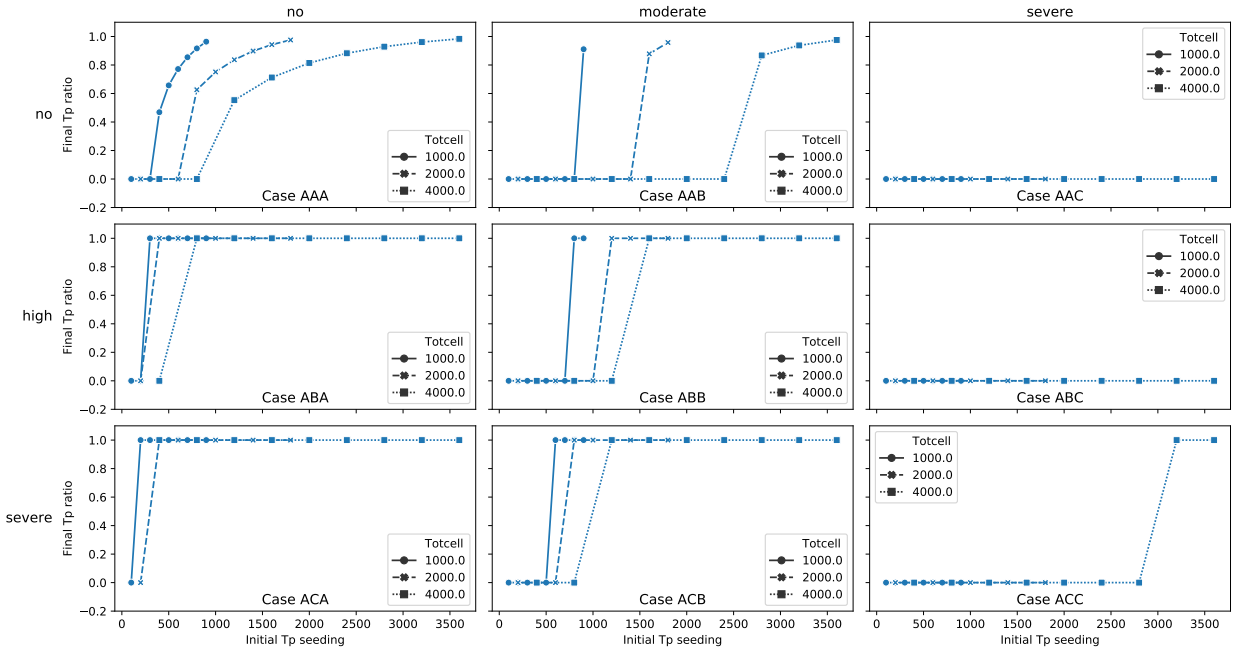
From the above observations, the following cases were formulated as an exhaustive formulation of possible conditions. Three levels of testosterone limitation of T^p pairwise competitive runs were done over varying initial cell seeding.

Case	O_2 **production**	T^- O_2 **limitation**	T^p *test* **limitation**
AAA	normal	no	no
AAB	normal	no	moderate
AAC	normal	no	severe
ABA	normal	high	no
ABB	normal	high	moderate
ABC	normal	high	severe
ACA	normal	severe	no
ACB	normal	severe	moderate
ACC	normal	severe	severe
BAA	poor	no	no
BAB	poor	no	moderate
BAC	poor	no	severe
BBA	poor	high	no
BBB	poor	high	moderate
BBC	poor	high	severe
BCA	poor	severe	no
BCB	poor	severe	moderate
BCC	poor	severe	severe

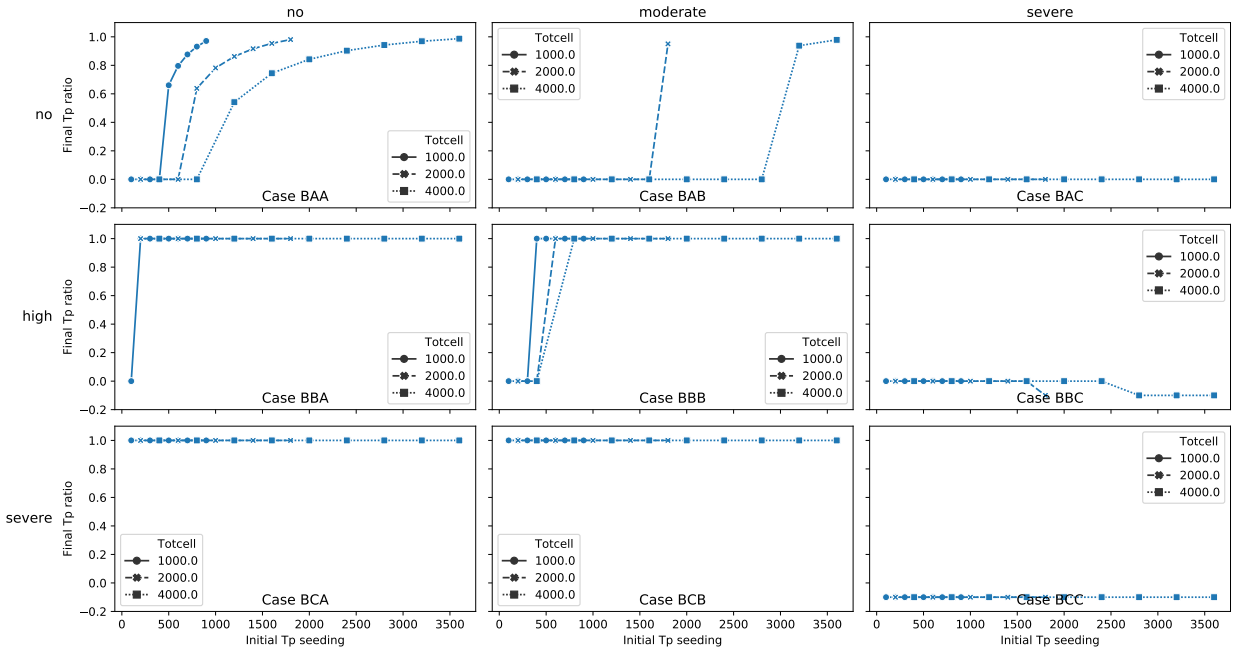
Table 3.1: Table of cases for T^p - T^- pairwise

Here,

- For O_2 production: normal and poor correspond to $p_{O_2} = 0.11, 0.0675$ min^{-1} respectively.
- For T^- O_2 limitation: no, high and severe correspond to $ll_{O_2,T^-} = 0, 0.6, 0.8$ respectively.
- For T^p *test* limitation: no, moderate and severe correspond to $ul_{test,T^p} = 0.1, 0.3, 1$ respectively.



(a) normal production



(b) poor production

Figure 3.4: 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 3.4:

1. Coexistence is observed only in cases AAA, AAB, BAA and BAB. All of them have low or moderate limitation of testosterone for T^p and have 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 shift towards T^p.

2. T^- causes T^p to go extinct for all initial seedings in cases AAC, ABC, BAC and BBC. T^p is severely testosterone limited and even with a high initial seeding advantage, T^- grows, overtakes T^p and eventually causes T^p to go extinct. At high T^p initial seeding, T^- also goes extinct in case BBC due to high oxygen limitation on it.

3. The outcome switches from T^p going extinct to T^- going extinct for higher T^p initial seeding in cases ABA, ABB, ACA, ACB, ACC, BBA and BBB. As with cases AAA, AAB, BAA and BAB, 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 shift 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. Case ACC has this switch only at the highest T^p initial seeding as it is also severely testosterone limited.

4. T^- goes extinct for all initial seedings in cases BCA, BCB and BCC. T^p also goes extinct in the case BCC. The oxygen limitation on T^- is too high and the oxygen levels never reach the levels required for a non-zero growth for T^-. In the case BCC, 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.

3.2 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 3.5.

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 3.6.

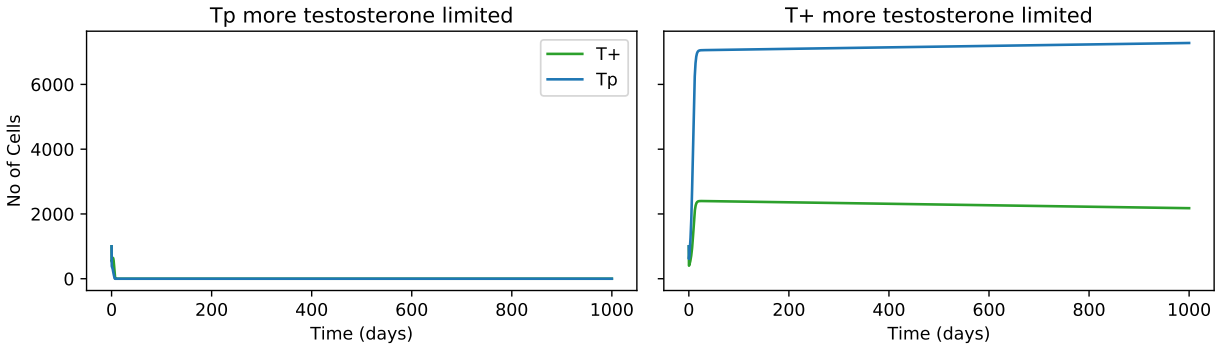


Figure 3.5: Pairwise $T^+ - T^p$ timeseries, when T^p is more testosterone limited than T^p and when T^+ is more testosterone limited than T^p. T^p is more limited testosterone limited at $ul_{test,T^+} = 0.3, ul_{test,T^p} = 0.5$ and T^+ is limited more at $ul_{test,T^+} = 0.5, ul_{test,T^p} = 0.3$.

From the above observations, the following cases were formulated as representative and pairwise competitive runs were done over varying initial cell seeding.

Case	T^+ *test* **limitation**	T^+ O_2 **limitation**	T^p *test* **limitation**	T^p O_2 **limitation**
1	no	no	moderate	no
2	moderate	no	no	no
3	severe	high	severe	moderate
4	severe	high	severe	no

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

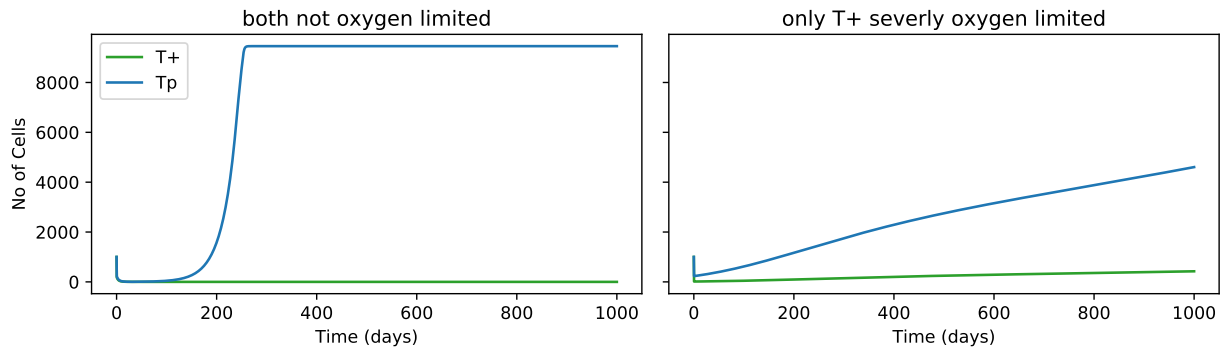


Figure 3.6: Pairwise $T^+ - T^p$ timeseries, when both cell types are testosterone limited and not oxygen limited at $ll_{O_2,T^+} = 0.0, ll_{O_2,T^p} = 0.0$ and T^+ is oxygen limited and T^p moderately at $ll_{O_2,T^+} = 0.6, ll_{O_2,T^p} = 0.4$.

Here, for T^+/T^p *test* limitation: no and moderate correspond to $ll_{O_2,T^i} = 0.1, 0.3$ respectively.

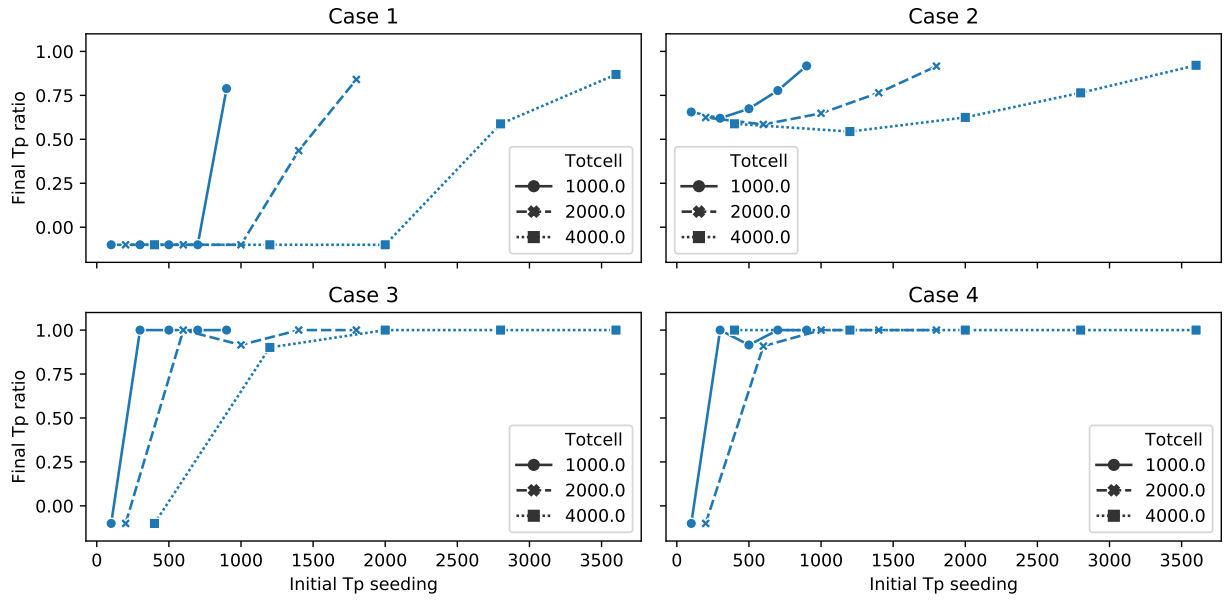


Figure 3.7: Final T^p ratio of pairwise $T^+ - T^p$ runs under different cases. Case 1: T^+ is not testosterone limited and T^p is moderately testosterone limited, Case 2: T^+ is moderately testosterone limited and T^p is not testosterone limited. Note: Ratio = -0.1 is used when both cell types go extinct.

The following were observed from the cases as visualised in Figure 3.4:

1. In case 1, both the cell types go extinct when T^p has low initial seeding as seen previously, whereas at high T^p initial seeding, both are able to coexist. At high T^p initial seeding, T^p is able to grow without density-dependent suppression by T^+ and secrete more testosterone from their higher overall number despite being disadvantaged in their growth compared to T^+.

2. In case 2, both cells are able to coexist at all T^p initial seeding. However, there exists a sweet spot for T^+ where it has the maximum final ratio. At high T^p initial seeding, T^p suppresses T^+ from growing through its large number, whereas, at very low T^p initial seeding, T^+ needs T^p to grow for its testosterone.

3. In case 3, at low T^p initial seeding, both cell types go extinct as T^+ due to it's higher number consumes the testosterone produced by T^p and doesn't let it grow. However, at higher T^p seeding, T^+ goes extinct as T^+ cannot grow due to oxygen limitation before being suppressed by T^p. Only at intermediate T^p seeding do both coexist as both of them inhibit each other for oxygen.

4. Case 4 is very similar to Case 3, however, the intermediate T^p seeding at which they coexist is lower. Since, T^p is not oxygen limited, it can grow more easily compared to Case 3 for the same initial T^p seeding and can outcompete T^+ more easily.

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