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Tesi di Laurea

Ecology of cancer: an evolutionary game theory approach to model cancer growth

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Introduction

Living systems are characterized by the emergence of regular statistical traits at various scale of magnitude. Many of such regularities are deemed to be somehow independent of the details of the system under consideration and to follow from few fundamental features. These observations hint at the presence of common basic mechanisms that may be described through simple mathematical models. The attempt of modeling such complex systems naturally leads to consider large families of microscopic identical units. Complexity and self-organization then arise on a macroscopic scale from the dynamics of these minimal components that evolve coupled by interaction terms. Indeed, there is an increasing evidence that one key feature of living systems lies in the architecture of their interaction networks. For example, this idea has proven to be useful to study the dynamics of complex ecosystems where a huge number of species (plants, animals etc...) coexist over a limited amount of resources. These ecosystems are characterized by multiple type of interactions (for instance competitive or mutualistic) that shape their dynamics and guarantee biodiversity maintenance. The same approach has been recently developed to study cell dynamics within a tissue. Cells that make up a complex organism - i.e. tissues or organs - can be seen as different species coexisting in a habitat. Habitat which offers limited resources, but that is considered relatively calm in the sense that it does not influence directly the survival of the various groups of cells. Therefore, the idea is to develop models that allow to investigate how different groups of cells interact and how interaction influences their dynamics in a specific environment.
Within this scenario, we are interested in applying such a framework for modeling tumor growth. There is the hope that quantitative predictions of disease development may suggest possible treatment strategies. This research direction seems to be promising and it is worldwide very active.

In the thesis we focus on multiple myeloma bone disease. In this cancer three types of cells are present. Among the different groups of cells, we will identify two groups of cells that we will call "residents" that are healthy cells normally present in the body -i.e. osteoblast (OB) and osteoclast (OC) -, and a third group of mutant malignant cells (MM) - i.e. the tumor - that we call "invaders" that tries to replace the osteoblasts. The aim is to analyze a deterministic model of the dynamics of the evolution of these three types of cells and explore the effects of different interaction networks among cell groups. Main theoretical tool for our analysis will be Evolutionary Game Theory (EGT).

We start with a biological introduction in order to briefly explain what myeloma bone disease consists of, and which relationships exist between the various cells that participate in the dynamics. Then we expose the mathematical tools and methods useful for our study. In particular the so-called replicator equation which is a fundamental object in EGT. The model we consider in the thesis takes explicitly into account the topology of a (simple) interaction network. Different topologies and/or different strengths of the interactions may lead to opposite outcomes: a cancer that invades the whole bone versus a cancer that disappear for a complete recover of the patient. We also consider the consequences of a transplant and drug therapies and we will show mathematically that it is impossible to completely eradicate the tumor through only the transplant and therefore an alternative cure is needed. These behaviors are studied in detail. Finally, we add a fourth species to our three-species model. The introduction of an additional

species has the following biological motivation: multiple myeloma is usually compound of different sub-clonal populations each having specific characteristics and all competing for the same available resources. Those that present better abilities to adapt will become the dominant sub-clone and so a targeted treatment may be directed against them. We will therefore study a system composed of two sub-clonal groups of mutants and the effect that a specific cure may have on them. We try to evaluate the goodness of the latter model and its predictions, its uses and possible therapeutic indications.

Chapter 1

The multiple myeloma bone disease

In this section we are going to explain the main features of the multiple myeloma bone disease. It occurs in the bone tissue and if not properly treated it can lead to serious consequences for the patient. The progression of the tumor, in fact, often induces a chronic fragility of the bones with consequent fractures and eventually, to death.

Multiple myeloma is a tumor characterized by the proliferation of MM cells in the bone marrow. The MM cells are malignant mutant cells that we will call invaders, which try to replace the normal types of cells already existing, that we will call residents. Therefore, it is of fundamental importance to understand the pathogenesis of this tumor, which is responsible for an increase in osteoclast activity and the inhibition of osteoblast activity, caused by interactions with malignant cells.

We start by explaining the role of the main types of cell that are normally present in a healthy organism's bone tissue [3]: osteoblasts (OB) and osteoclasts (OC). Together with the osteocytes they regulate the bone's remodeling. This is a process consisting of a well-balanced relationship between reabsorption and bone formation. The first activity is regulated by the osteoclasts, while the second is due to osteoblasts. Osteocytes, on the other hand, make up 95% of bone cells and play a fundamental role in bone remodeling by regulating both osteoblast and osteoclast activity.
Figure 1.1 below represents the many interactions that we can observe in a normal situation, before the arising of the cancer. Each arrow represents an interaction that stimulates the development of the indicated cells. Starting from the OB cells, we move to the precursors of the OC, which will become OC, to pass then to the osteoblasts' precursors and return. This path represents the cycle of interactions that guarantees a correct homeostasis. These interactions (the one involving RANKL / OPG in particular) will be described in more details later when we will introduce MM cells.

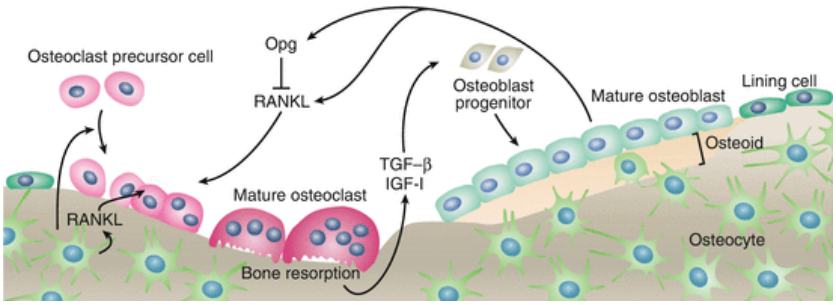


Figure 1.1: The normal intercellular interactions [10]

Therefore, both in normal situations and after the MM appearance, osteoblasts and osteoclasts pos-

itively interfere with each other, inducing a dynamic which we will explore in section 2.1. This result leads us to a substantial balance between OC and OB cells. The homeostasis will be studied in detail below when we will see a first example of an application of game theory. By assuming positive interactions between these cells, we will be able to verify their coexistence and how together they regulate the normal physiology of the bone.

Let us introduce the many interactions generated due to the introduction of MM cells, which we assume to have already been formed. The interactions between the various groups of cells are multiple and complex. Below is a summary diagram that we will analyze in detail (Figure 1.2) and which shows the different types of cells that are present. In addition to those already mentioned, in fact, there are the bone marrow stromal cells (BMSC), the precursor of osteoclasts and Th-17.

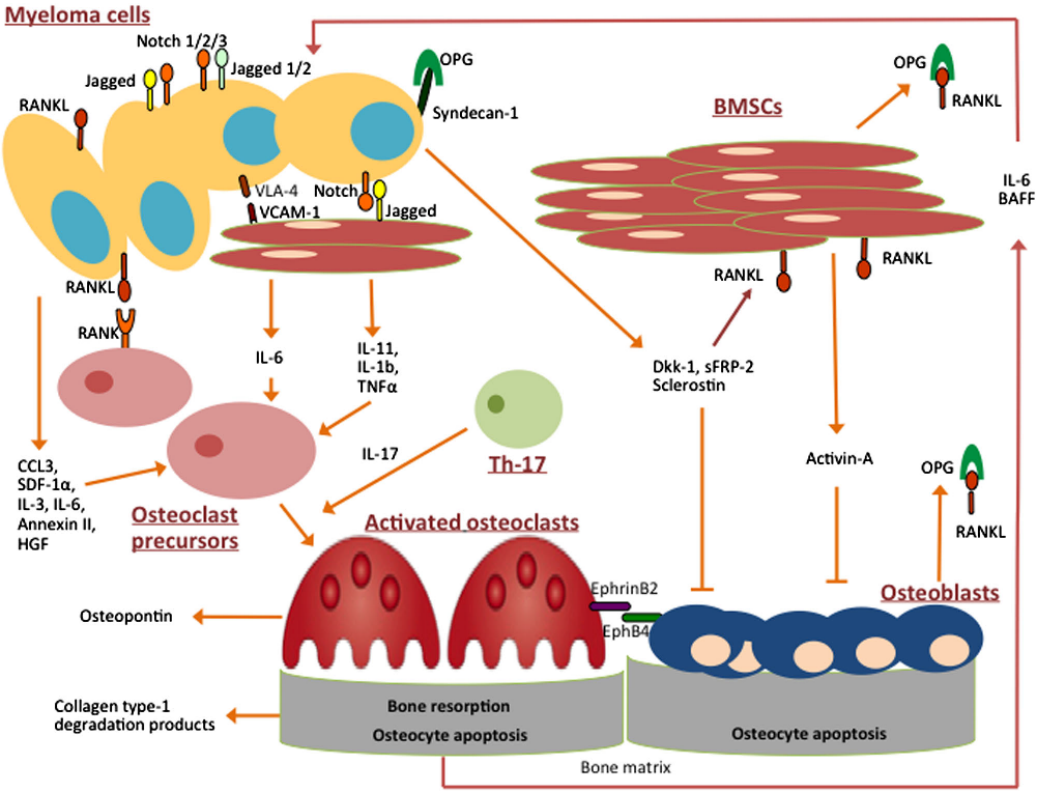


Figure 1.2: The intercellular interactions with MM cells [3]

Looking at Figure 1.2, it can be noticed that the interaction between the malignant MM cells and the BMSC cells with the participation of immune cells such as Th17, leads to the release of cytokines such as IL-1b, IL-3, IL-6, IL-11, IL-17. These latter play different roles:

- Interleukin IL-3 cytokines have a double function: they inhibit osteoblast differentiation and induce osteoclast formation. High levels of these Interleukins have been detected in patients suffering from this disease;
- Interleukin IL-6 stimulates the differentiation of OC cells;
- Interleukin IL-17 secreted by T-helper cells (Th17) promotes activation of OC cells and simulta-

neously causes osteolytic lesions.

The mutant cells secret factors such as TNF-α, CCL-3, SDF-1α, and annexin II in the microenvironment:

- high TNF-α levels are present in patients affected by this tumor and they promote osteoclastogenesis;
- chemokine (C-C motif) ligand3 (CCL-3) promotes the formation of OC cells by attracting their precursors and simultaneously inhibits osteoblast activity;
- SDF-1α promotes osteoclast activity;
- Annexin II stimulates osteoclastogenesis.

The increase in osteoclast activity is also due to the RANK receptor. It is a transmembrane receptor that could be found in the precursor cells of the osteoblasts. It binds with RANKL which is a cytokine normally present in the BMSC cell membrane. When this binding occurs, a fusion of the osteoclast's precursor cells is induced, leading to the formation of multinuclear cells that will become the osteoclasts.
Osteoprotegerin (OPG) is produced by osteoblasts and BMSCs. It is the antagonist of RANK. So, binding with RANKL, inhibits the formation of osteoclasts. In this way, the resorption of bone by osteoclasts is checked.

However, when the mutant cells intervene in the system, they perform a series of processes:

- the expression of Notch which binds with Jagged produced by the adjacent MM cells and by the BMSCs, which causes a cascade reaction that culminates in the production of RANK;
- induced apoptosis of cytokines in which another RANKL is released;
- Syndecan-1 production, through which myeloma cells bind, internalizes and degrades OPG produced by osteoblasts.

These combined actions increase the production of RANKL and inhibit that of OPG, completely disrupting the normal regulation chain of these processes, in favor of an uncontrolled formation of osteoclasts.
The osteoclasts also produce Interleukin IL-6 and BAFF that improve the growth, sustaining and survival of MM cells.
Moreover, MM cells produce factors such as DKK1, sFRP-2, and sclerostin that directly inhibit Wnt3a whose action is to regulate osteoblast differentiation thus reducing the expression of OPT and altering the process developed between OPG and RANKL described above.
Finally, BMSC cells produce Activin-A which inhibits the formation of osteoblasts and activates osteoclasts instead.

In order to implement a mathematical description, the process must be simplified. Therefore, we take into account only the cells positioned at the end of the processes' chain.
Therefore, we ignore the other types of existing cells and the many interactions and effects that they have on the main cells. We only consider osteoblasts (OB), osteoclasts (OC) and malignant cells (MM). Through this simplification we obtain a dynamics that concerns only these three types of cells, which however sums up the main characteristics of the cancer evolution.

Following this path, we summarize the various interactions in 5 classes of relationships between groups of cells (on the right of each arrow we highlight the parameter that will be associated to the relation, once mathematically modeled), such as:

1. OB cells positively stimulate OC cells $\longrightarrow \alpha$;
2. OC cells positively stimulate OB cells $\longrightarrow \varepsilon$;
3. MM cells positively stimulate OC cells $\longrightarrow \beta$;
4. OC cells positively stimulate the MM cells $\longrightarrow \gamma$;
5. MM cells negatively stimulate OB cells $\longrightarrow -\delta$;

OB cells, on the other hand, have no effect on MM cells.

Therefore, these interactions are able to increase the activity and production of osteoclasts, inhibit that of the ostoblasts and their differentiation, with the consequent growth of the tumor cells. The result is thus the reabsorption and destruction of the bone, leading to multiple fractures and damages. The simplified model is shown in Figure 1.3.

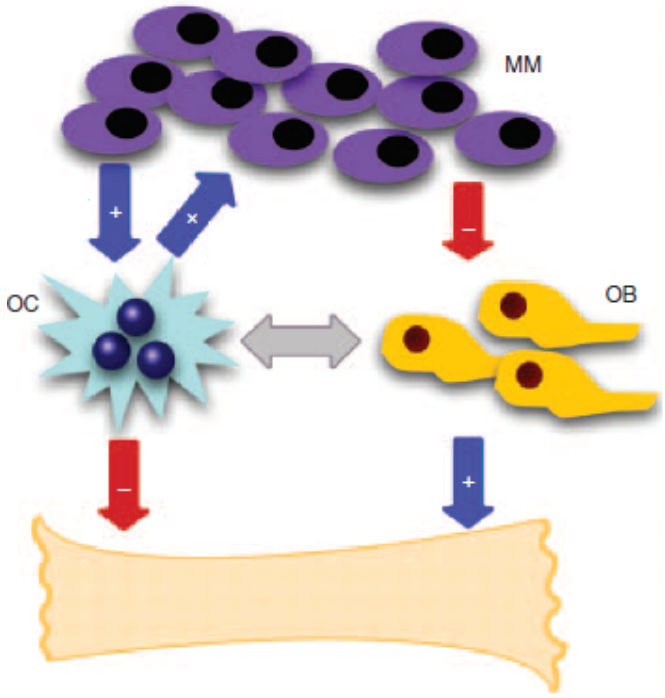


Figure 1.3: The simplified intercellular interactions [2]

In this mere simplification of the original interactions we neglect the presence of possible sub-populations in the tumor population (e.g., myeloma stem cells, sub-clones...). We will discuss the implication of this choice in the last part of this thesis.

Chapter 2

The model

The model [1] [2] we are going to study is based on evolutionary game theory and its application to living ecosystems. A brief introduction on these mathematical concepts is given in the appendix.
With the term ecosystem we indicate a set of sundry living and non-living organisms that interact with each other. These relationships existing between its various components drive the evolution of the several species belonging to the ecosystem. They are many and of different nature. What regulate the number of individuals of a certain species in a complex system are the rates of birth and death. These represent two main characteristics that a living being possesses and are influenced by the interactions that the species have among themselves and with the external environment. To fully understand how an ecosystem may evolve in time, is then necessary to identify the relationships that exist between its several components and to model them. This means to consider the effects and to simplify them, in order to obtain a description based on a low number of parameters, but that keeps a good level of predictive power.

The idea behind the model we wish to propose is to treat the organism as a large ecosystem, whose different species are the many types of cells of which it is compound. In fact, they have very diversified roles within the organism and moreover, multiple relationships are present between them and with the external environment. As the various species of the ecosystem, they unknowingly play their role in keeping the organism healthy by carrying out the tasks for which they have evolved. Different roles may also be in contrast with each other like that of OB cells and OC cells, but both are fundamental for the ecosystem maintenance. Finally, each individual can give birth to a similar cell or die.
Our body is made up of cells living in an environment that provides them with sustenance and that they help keeping alive. In return they are able to reproduce, i.e. generate an exact copy of themselves. This process occurs regularly and is strongly controlled by the presence of other cells and by the environment itself.

Our derivation of the replicator equation from the exponential one fits well the model for cancer cells.
In fact, at first we can consider that cells replicate at a constant rate. The equation that rules the process is therefore an exponential law:

$$\dot{N}_i = \alpha_i N_i \tag{2.1}$$

that gives:

$$N_i(t) = N_n(0) e^{\alpha_i t}, \tag{2.2}$$

where N_i is the number of cells and α_i is the replication rate for type i cell. However, this is a very unrealistic scenario in which the population sizes of all cellular types increase exponentially.

A more realistic one imposes a limit on the available resources, that enforces the constancy of the total size of the population.
This is the reason why we introduce the replicator equation. We wish to study the evolution of the disease, treating cells as different species interacting with each other and therefore following an evolutionary dynamics.

In order to do this in our model, we have to consider that cells can interact with every other cell: this is a well known approximation called well-mixed approximation. This hypothesis is based on the fact that cells have an average life that allows them to interact with many others. Furthermore, they must be able to walk great distances before dying, so that they are become in contact with an average number of individuals that represents the entire population at all times. Thus, cells are considered free to move at high speed compared to their average life.

Currently, we assume that, during the evolution of the tumor dynamics, no other mutations occur, apart from the one that gave rise to the mutants. The idea is consider a system where the mutant cells have been already completely developed, i.e. when all the mutations necessary for a cell to become a tumor have already taken place. This hypothesis is necessary in our first model, as we will only consider three groups of cells. However this constraint will be relaxed in the future, when we study the development of a sub-clonal population with a different set of mutation.

Another basically approximation is to neglect the possible stochastic behaviors that cells may assume giving rise to a further variability of the disease. Although there are many reasons why an analysis should be made from a stochastic point of view (the cells may present different probabilities to undertake an interaction rather than another, death could be caused by factors other than simple lack of resources, the external environment could randomly bring benefits such as oxygen and nutrients and many other causes that we do not list). Our aim is to investigate the process from a deterministic perspective (let us notice that this hypothesis is partly weakered by the mean-field approximation, which attributes the same probability a priori to each interaction between the various types of cells).

Furthermore, if we wish to develop an evolutionary model for cancer cells, and thus to introduce the replicator equation, we have to assume that population sizes of the various types of cells are large enough to convert the number of cells N_i into frequencies x_i.
Hence, the constancy of the cells' number reflected itself on the constancy of the sum of the frequency, which can be written like:

$$\sum_i x_i \equiv 1 \tag{2.3}$$

2.1 Normal bone remodelling

This first model describing homeostasis allows us to verify, for a simple case, the method we will later apply to more general cases.
We begin modeling the normal balance between OC cells and OB cells. This is the situation that occurs before the onset of the tumor. It can be analyzed in the context of a trivial EGT.

What we expect is that the frequencies of the two groups of healthy cells converge after sufficient time to a stable point. In fact, in healthy body conditions, neither group can overpower the other, resulting in the coexistence of the two types of cells that positively influence each other. The only negative effect that they suffer, which limits their proliferation, is given by the surrounding environment, which provides a limited amount of resources. Thus, cells are naturally conducted towards the number that optimizes their work within the body. Our simple model easily explains how our body maintains the conditions of homeostasis within the bones.

We have two types of cells whose interaction can be modeled by the following payoff matrix:

$$\begin{array}{c} \\ OC \\ OB \end{array} \begin{array}{c} \begin{array}{cc} OC & OB \end{array} \\ \begin{pmatrix} 0 & \alpha \\ \varepsilon & 0 \end{pmatrix} \end{array} \tag{2.4}$$

where α and ε are the payoff associated with the mutual interaction between the two types of cells. For example α represents the payoff earned from an OC cell when it interacts with an OB cell. They are positive because the effect of each group of cells on the other is an incentive to growth; in fact we are limiting ourselves to model the first 2 groups of interactions of the 5 described in the chapter 1, since in this case we are only interested in those between the OB and OC cells.
We refer to the appendix for the derivation of the replicator equation starting from the exponential previously introduced model. Let us use it directly here where x represents the concentration of the OC cells and $\dot{x}$ its time derivative:

$$\dot{x} = x(1-x)(\alpha(1-x) - \varepsilon x) \equiv f(x) \tag{2.5}$$

which presents three fixed points in:

$$x = 0, \tag{2.6}$$

$$x = 1 \tag{2.7}$$

and

$$x = \frac{\alpha}{\alpha + \varepsilon}. \tag{2.8}$$

Populations composed of one type of cells - i.e. (2.6) and (2.7) - are unstable, while (2.8), which presents both OC and OB cells, is stable. To prove it, we resort the one dimensional version of the Lyapunov method (the theorem is stated in the appendix). Deriving the replicator equation, we obtain:

$$\frac{df(x)}{dx} = \varepsilon x(-2+3x) + \alpha(1-4x+3x^2) \tag{2.9}$$

Evaluating it in its three fixed points $x = 0$, $x = 1$ and $x = \frac{\alpha}{\alpha+\varepsilon}$ we get, respectively:

$$f(0) = \alpha > 0 \tag{2.10}$$

$$f(1) = \varepsilon > 0 \tag{2.11}$$

$$f(\frac{\alpha}{\alpha+\varepsilon}) = -\frac{\alpha\varepsilon}{\alpha+\varepsilon} < 0 \tag{2.12}$$

This proves our thesis, where the eigenvalues are shown.
Below we show some explanatory graphs.

This first graph (Figure 2.1) represents the evolution of the concentration of the two types of cells over time. The convergence to the equilibrium that represents an ESS is highlighted. This rest point corresponds exactly to the perfect subdivision between the two groups of cells, due to the particular choice of parameters, that is $\alpha = \varepsilon = 0.5$. Not necessarily, this peculiar situation recurs in a real case, but the dynamic maintains the same features shown here, since is derived from the general replicator equation (2.5).

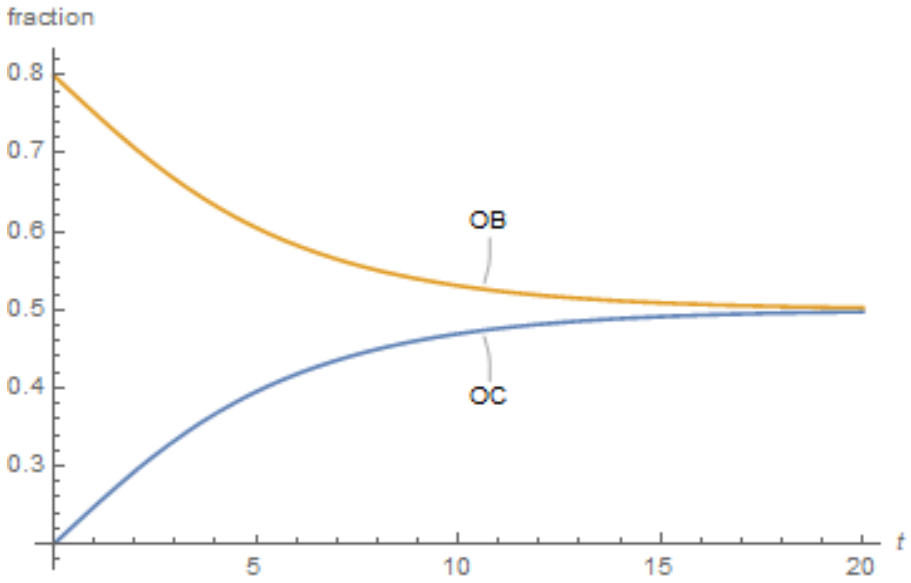


Figure 2.1: α=0.5; ε=0.5; starting point: (OC,OB)=(0,2; 0,8)

The second graph (Figure 2.2) shows the derivative of the frequency of the OC cells and therefore their variation over time, in a range between 0 and 1, that is the frequency domain. The stable and unstable equilibrium points found before are indicated.

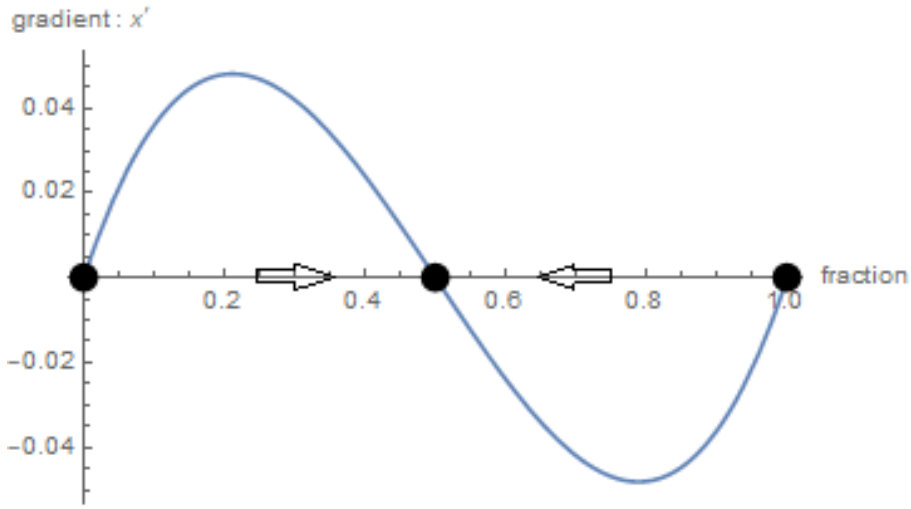


Figure 2.2: α=0.5; ε=0.5

Any deviation from stable equilibrium leads to the development of a dynamics suitable for restoring stability. This means that, under normal conditions, cells are able to tune their quantity to return to the best condition. There is no evolutionary battle here. The cells coexist and perform their tasks to keep the organism healthy and efficient.

2.2 MM cells

Let us analyze now how the situation change when mutant cells intervene in the evolutionary game. Mutant cells are seen as invaders seeking to replicate in the new ecosystem and supplant resident cells. In fact, they are longing to replace the osteoblasts with a consequent serious damage to the bone. What emerges is the establishment of a real hunger game between cells. Indeed, the advent of MM causes strong changes in the usual activity of healthy cells, as previously described. The struggle for survival, as it happens in biological ecosystems, is based on the interactions they have with each other. Cancer cells are endowed with excellent characteristics useful for introducing and proliferating. Only an adequate development of OB cells will allow them to survive. Therefore the role of the evolutionary game theory, firstly developed for ecology, becomes clear in this model, that at first sight could have seemed purely biological.

In agreement with the hypotheses exposed before, we will use the simplified scheme of interactions. We will then introduce the replicator equation applied to the three different groups of cells, and we will write the payoff matrix in which the various interactions are parametrized.
By combining the two, we will be able to write the differential equations that regulate the dynamics of all the cells. We will find the fixed points of this system and evaluate its stability through the Lyapunov theorem previously stated. From the analysis of the stability we will obtain important information about the evolution of the system in the different cases we study, which will be, subsequently, treated numerically.

The replicator equation

We can write the replicator equations which rule the dynamics for our three types of cells, we refer to the appendix for their derivation:

$$\dot{x_i}(t) = x_i(t)(F_i(x_1,x_2,x_3) - < F >) \tag{2.13}$$

where

$$F_i(x_1,x_2,x_3) = \sum_{k=1}^{3} A_{ik}x_k \tag{2.14}$$

and

$$< F >= \sum_{i=1}^{3}\sum_{k=1}^{3} x_i A_{ik} x_k. \tag{2.15}$$

Matrix **A** in (2.14) and (2.15) is the payoff matrix, whose elements represent the interaction between several types of cells. We have thus replaced the function that represents the fitness of the strategies by a linear model based on a payoff matrix.

Its domain is given by:

$$S := \left\{ (x_1,...,x_n) \in \mathbb{R}^n : \sum_{i=1}^{n} x_i = 1,\ \ x_i \geq 1,\ \ i = 1,...,n \right\}. \tag{2.16}$$

We easily see that it is invariant [8] - in the sense of (2.3) - for the RE :

$$\frac{d}{dt}\left(\sum_{i=1}^{n} x_i\right) = \sum_{i=1}^{n} \frac{dx_i}{dt} = \sum_{i=1}^{n} x_i (Ax)_i - \underbrace{\sum_{i=1}^{n} x_i}_{1} \sum_{j=1}^{n} x_j (Ax)_j = 0 \tag{2.17}$$

Therefore, as shown above, the replicator equation forces the dynamics of the ecosystem within the simplex: in each point the sum of cell frequencies is constant. This means that the previously introduced hypothesis of the constancy of the total population (based on available resources) is made a priori by the replicator equation and does not require other specific manipulations. Moreover this derivation is independent from the dimension and it will therefore remain effective when we introduce the four-cells model.
The next step is to introduce the payoff matrix that characterizes our particular model, and identifying the fixed points of the dynamics. Once obtained, we will evaluate their stability to understand which strategy is evolutionary stable (ESS).

The payoff matrix

The fundamental concept of game theory is the payoff matrix, as we explain in the appendix. The rows of this matrix represent the specific group of cells we are considering. Instead, the columns indicate the type of cells they are interacting with. The matrix elements thus indicate the effect that cells in the columns have on those in the rows

$$\mathbf{A} = \begin{array}{c} \\ OC \\ OB \\ MM \end{array} \begin{array}{c} \begin{array}{ccc} OC & OB & MM \end{array} \\ \begin{pmatrix} 0 & \alpha & \beta \\ \varepsilon & 0 & -\delta \\ \gamma & 0 & 0 \end{pmatrix} \end{array} \tag{2.18}$$

Relying on the true biological relationships between cells, we can assign to each interaction a parameter of this matrix, which represents the advantage/disadvantage that a cell receives when it is put in contact with one of a different type. In contrast, interactions between cells of the same type are assumed to be neutral.
We set all the parameters to be positive, so that, if an interaction creates advantage, we maintain the plus sign, however, whereas if it comes to be disadvantageous for a group of cells (between MM and OB for example), we add a minus sign.

Let us prove that, thanks to the properties of the replicator equation combined with EGT, we can greatly simplify the form of this matrix, without modifying the results.

Proposition 2.2.1. *The new replicator equation:*

$$\dot{y}_i = \left(y_i \sum_{k=1}^{n} B_{ik} y_k - y_i \sum_{j=1}^{n} \sum_{k=1}^{n} y_j B_{jk} y_k \right) \tag{2.19}$$

obtained from the RE in (2.13) with following transformation:

$$y_i = \frac{x_i \phi_i}{\sum_{j=1}^{3} x_j \phi_j} \tag{2.20}$$

is equivalent to the original one, in the sense that stability/instability properties of the fixed points should be guaranteed but not their positions, where $\mathbf{A}$ *matrix will be replaced by* $\mathbf{B}$ *matrix with* $B_{ij} = \frac{A_{ij}}{\phi_j}$.

Proof. Suppose that x_i with $i = 1, ..., n$ satisfy the replicator equation with matrix A. Then:

$$\frac{d}{dt}y_i = \frac{\dot{x}_i\phi_i}{\sum_{j=1}^{n} x_j\phi_j} - \frac{x_i\phi_i}{\left(\sum_{j=1}^{n} x_j\phi_j\right)^2}\left(\sum_{j=1}^{n}\dot{x}_j\phi_j\right) \tag{2.21}$$

let us denote with $c \equiv \sum_{j=1}^{n} x_j\phi_j$.

Using the RE: $\dot{x}_i(t) = x_i(t)\left(\sum_{k=1}^{n} A_{ik}x_k - \sum_{h=1}^{n}\sum_{k=1}^{n} x_hA_{hk}x_k\right)$, (2.21) becomes:

$$\frac{d}{dt}y_i = y_i\sum_{k=1}^{n} A_{ik}x_k - y_i\sum_{h=1}^{n}\sum_{k=1}^{n} x_hA_{hk}x_k - \frac{y_i}{c}\left(\sum_{j=1}^{n} x_j\phi_j\sum_{k=1}^{n} A_{ik}x_k - \sum_{j=1}^{n} x_j\phi_j\sum_{h=1}^{n}\sum_{k=1}^{n} x_hA_{hk}x_k\right) \tag{2.22}$$

Using (2.20):

$$\frac{d}{dt}y_i = y_i\sum_{k=1}^{n} A_{ik}x_k - y_i\sum_{h=1}^{n}\sum_{k=1}^{n} x_hA_{hk}x_k - y_i\left(\sum_{j=1}^{n} y_j\sum_{k=1}^{n} A_{jk}x_k - \underbrace{\sum_{j=1}^{n} y_j}_{1}\sum_{h=1}^{n}\sum_{k=1}^{n} x_hA_{hk}x_k\right) \tag{2.23}$$

Since $\sum_{j=1}^{n} y_j = 1$, we can cancel out the second and the last term of 2.23, and we obtain:

$$\dot{y}_i = y_i\sum_{k=1}^{n} A_{ik}x_k - y_i\sum_{j=1}^{n}\sum_{k=1}^{n} y_jA_{jk}x_k. \tag{2.24}$$

Multiplying and dividing 2.24 by ϕ_k and c the terms of the sums:

$$\dot{y}_i = y_i\sum_{k=1}^{n}\underbrace{\frac{A_{ik}}{\phi_k}}_{B_{ik}}\underbrace{\frac{x_k\phi_k}{c}}_{y_k}c - y_i\sum_{j=1}^{n}\sum_{k=1}^{n} y_j\underbrace{\frac{A_{jk}}{\phi_k}}_{B_{jk}}\underbrace{\frac{x_k\phi_k}{c}}_{y_k}c \tag{2.25}$$

And thus:

$$\dot{y}_i = \left(y_i\sum_{k=1}^{n} B_{ik}y_k - y_i\sum_{j=1}^{n}\sum_{k=1}^{n} y_jB_{jk}y_k\right)c. \tag{2.26}$$

This is the replicator equation but for a c-factor, which can though be erased by rescaling the time $\tilde{t} = \frac{t}{c}$.

□

In the specific case: $(\phi_1, \phi_2, \phi_3) = (\varepsilon, \alpha, \frac{\beta\varepsilon}{\gamma})$:

$$\mathbf{B} = \begin{array}{c} \\ OC \\ OB \\ MM \end{array}\begin{array}{c} \begin{array}{ccc} OC & OB & MM \end{array} \\ \begin{pmatrix} 0 & 1 & b \\ 1 & 0 & -d \\ b & 0 & 0 \end{pmatrix} \end{array} \tag{2.27}$$

where $b = \frac{\gamma}{\varepsilon}$ and $d = \frac{\delta\gamma}{\beta\varepsilon}$.

This new payoff matrix generates a system completely equivalent to that generated by matrix **A**. However, it gives rise to much simpler equations, and above all, we can study the evolutionary dynamics based on two parameters which completely characterize the tumor.
On the other hand, it is important to stress out that these parameters can not be derived from an analysis of the newly constructed model. They vary from one clinical case to another.

Using the **B** matrix in the replicator equation, we obtain:

$$\begin{cases} \dot{x}(t) = x(y+bz-2xy-2bxz+dyz) \\ \dot{y}(t) = y(x-dz-2xy-2bxz+dyz) \\ \dot{z}(t) = z(bx+bz-2xy-2bxz+dyz) \end{cases} \tag{2.28}$$

where in (2.28) x represents the concentration of type OC cells, y of the OB cells and lastly z of the MM cells.

Fixed points

Let us now look for the fixed points of the dynamic system defined in equation (2.28). These, will allow us to understand how the solution evolves. We can find them by imposing the stationary condition:

$$\begin{cases} x(y+bz-2xy-2bxz+dyz) = 0 \\ y(x-dz-2xy-2bxz+dyz) = 0 \\ z(bx+bz-2xy-2bxz+dyz) = 0 \end{cases} \tag{2.29}$$

from (3.2) we obtain:

$$(x,y,z) = (0,0,0) \tag{2.30}$$

$$(x,y,z) = (0,0,1) \tag{2.31}$$

$$(x,y,z) = (0,1,0) \tag{2.32}$$

$$(x,y,z) = (1,0,0) \tag{2.33}$$

$$(x,y,z) = \left(\frac{1}{2},\frac{1}{2},0\right) \tag{2.34}$$

$$(x,y,z) = \left(\frac{1}{2},0,\frac{1}{2}\right) \tag{2.35}$$

$$(x,y,z) = \left(\frac{d}{1-2b+b^2+d+bd}, \frac{b(-1+b+d)}{1-2b+b^2+d+bd}, \frac{1-b}{1-2b+b^2+d+bd}\right) \tag{2.36}$$

The existence conditions of these fixed points are

$$x+y+z = 1, \tag{2.37}$$

and

$$\begin{cases} x \geq 0 \\ y \geq 0 \\ z \geq 0 \end{cases} \tag{2.38}$$

because the number of cells is constant and negative frequencies of cells are not allowed.
The first fixed point (2.30) has no reason to be in this model because it does not satisfy (2.37). We need to check when the last zero (2.36) should have the three components positive in the meantime (2.38). As we know from the definition, the parameters d and b are positive so considering the first component:

$$\frac{d}{1-2b+b^2+d+bd} \geq 0 \Leftrightarrow 1-2b+b^2+d+bd \geq 0. \tag{2.39}$$

Regarding the other two components and the obtained result (2.39), it shows that they could satisfy (2.38) when their numerators are positive. This fact involves that $b(-1+b+d) \geq 0 \Leftrightarrow b+d \geq 1$ for the second component of (2.36) and $b \leq 1$ for the third. Therefore, this fixed point exists only when the following needed conditions are together satisfied:

$$\begin{cases} 1-2b+b^2+d+bd \geq 0 \\ b \leq 1 \\ b+d-1 \geq 0 \end{cases} \tag{2.40}$$

The others fixed points do not need any check because they satisfy both (2.37) and (2.38). Therefore they always exist.

2.3 Stability analysis

The analysis of the stability is fundamental to find the evolutionarily winning strategies on a case by case basis, i.e. the evolutionary stable strategy.

In Appendix, we calculate the Jacobian matrix of the vector field associated to the differential system: i.e. we linearize the system in order to get information about the stability of these equilibria. Then, we can evaluate the Jacobian matrix in the fixed points that we found before.

Trivial equilibrium

As we show in the appendix, the vertex of the simplex, i.e. the fixed points that present only a monotypic population, that is a population with 100% cells of one type, are unstable: any initial data close enough to them goes indefinitely away from them.
Each of these, in fact, possesses at least one eigenvalue with a positive real part, as we can see in Table 2.1.

fixed point (x,y,z)	eigenvalues $(\lambda_1,\lambda_2,\lambda_3)$
$(1,0,0)$	$(1,0,b)$
$(0,1,0)$	$(1,0,0)$
$(0,0,1)$	$(0,b,-d)$

Table 2.1: Fixed points and respective eigenvalues

Boundary fixed points

The first, (2.34), represents the defeat of the cancer, as the frequency of MM cells is equal to zero and its eigenvalues are:

$$(\lambda_1, \lambda_2, \lambda_3) = \left(-\frac{1}{2}, -\frac{1}{2}, \frac{1}{2}(-1+b) \right). \tag{2.41}$$

It has two negatives eigenvalues and one which it is negative only when $b < 1$. Thus we have an asymptotically stable equilibrium and therefore an ESS.

Instead, the second (2.35), represents the death of the OB cells and the victory of the tumor, the eigenvalues are:

$$(\lambda_1, \lambda_2, \lambda_3) = \left(-\frac{b}{2}, -\frac{b}{2}, \frac{1}{2}(1-b-d) \right). \tag{2.42}$$

It has two negatives eigenvalues when $b > 0$ and one which results negative when $b > 1$ or $b < 1$ and $b + d > 1$. In this case we have an asymptotically stable point and so an ESS.

Interior fixed point

The last equilibrium point (2.36) that we are going to deal with is the one lying inside the simplex and therefore having all the three coordinates different from zero. Its eigenvalues are:

$$(\lambda_1, \lambda_2, \lambda_3) = \left(\frac{bd}{1+d+b(-2+b+d)}, -\frac{bd+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)}}{2(1+d+b(-2+b+d))}, \frac{-bd+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)}}{2(1+d+b(-2+b+d))} \right)$$

This must be a saddle point, because the second and the third eigenvalue present a opposite sign. In fact they share the same denominator whereas different numerators. The first:

$$-\left(bd + \sqrt{-bd\left(4(-1+b)^2 + (-4+3b)d\right)} \right) < 0$$

is always negative, whereas the second is always positive. Indeed,

$$-bd\left(4(-1+b)^2 + (-4+3b)d\right) > (bd)^2 \Leftrightarrow -\left(4(-1+b)^2 + (-4+3b)d\right) > bd \Leftrightarrow$$

$$-4 - 4b^2 + 8b - 3bd + 4d - bd > 0 \Leftrightarrow 1 + b^2 - 2b + bd - d < 0 \Leftrightarrow$$

$$(1-b)^2 < d(1-b) \Leftrightarrow \begin{cases} 1-b < d \\ b < 1 \end{cases}$$

Where we discard the solution with $b > 1$ that implies $d < 0$. Thus, it is positive if and only if the following system is satisfied :

$$\begin{cases} b < 1 \\ b + d > 1 \end{cases}$$

which are exactly the existence conditions of this fixed point (2.40).

2.4 Results

We show now some results where different values for b and d are used. Figures 2.3 and 2.5 are organized as follows. On the left we plot a 2D-graphic, the horizontal axis represents the concentration of OC cells, while the vertical one is the OB type frequency. The origin of the axes, is the point of maximum concentration of the MM cells, since we have $z = 1 - x - y$. We can think it as a projection on one of the three planes ($z = 0$ in this case) of the three-dimensional graphic, in which all three cell frequencies would be shown in each axis. Each vertex of the triangle represents a monotypic population, i.e. having all the cells of the same type. However this limit case is unattainable. Our discussion clears up that these points are unstable equilibria of the replicator dynamics.

In the graphics we indicate the several equilibria with different colors:

- Green $\longrightarrow$ stable point;
- Red $\longrightarrow$ unstable points;
- Gray $\longrightarrow$ saddle point.

Moreover, the colors of the simplex represent the velocities of the changes in the cellular frequency, according to the scale next to the graphics.
On the right we dot the temporal evolution of the three cellular frequencies once set a fixed initial point.

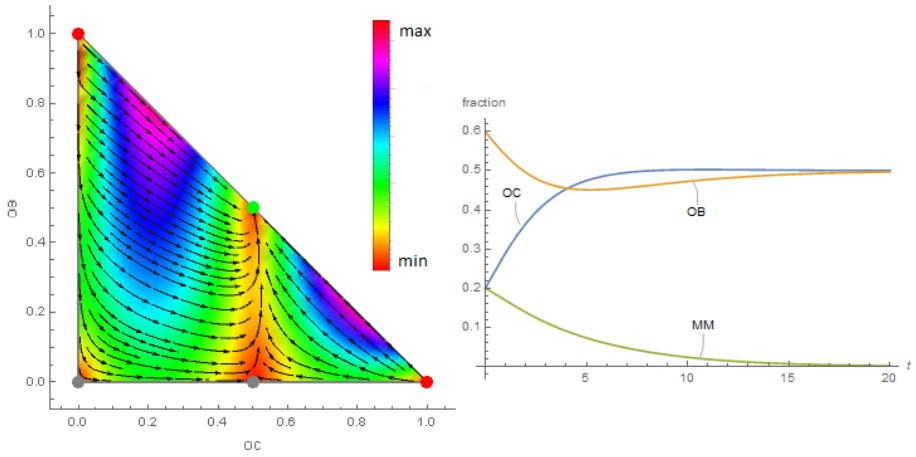
We choose to represent three different situations that summarize the different cases that may happen using (2.41) and (2.42), because of the fixed points:

- $b < 1 \wedge b + d < 1 \Longrightarrow$ (2.34) is asymptotically stable, (2.35) is unstable;
- $b < 1 \wedge b + d > 1 \Longrightarrow$ both (2.34) and (2.35) are asymptotically stable;
- $b > 1 \Longrightarrow$ (2.41) is unstable, (2.35) is asymptotically stable.

Of the three proposed cases, the last one corresponds to a developed cancer. Instead the first two represent more favorable situations that we hope to achieve with adequate therapy. For all the simulations we use the same set of starting coordinates (except in a special case that we will discuss below).
As expected, we will see that the velocity decreases when we approach a stable equilibrium because of the asymptotic convergence, resulting in a very slow implementation, since the effective extinction of a group of cells is unattainable.

Case $b = 1/2$, $d = 1/3$

The two graphs in Figure 2.3 show the result for $b = 1/2$ and $d = 1/3$, i.e. when the advantage brought by the interaction of mutant cells with healthy OC cells, is weak (less than those with OB) and when those mutants bring a slight disadvantage to OB cells. In particular $b + d < 1$ and $b < 1$.
It has been pointed out that the only point of stability of the dynamic belongs to the edge of the simplex, where we have the coexistence of OC and OB cells. Therefore the tumor will be extinguished.



(a) (b)

Figure 2.3: $b = 1/2; d = 1/3$; starting point: $(x, y, z) = (0.2, 0.6, 0.2)$

Here we highlight the result we obtained analytically: the b parameter is less than 1, therefore the OC cells interact in a stronger way with the osteoblasts with respect to the MM cells. Cancer cells did not obtain a complete set of mutations when we analyze the situation, that allows them to be more competitive and therefore change the dynamics in their own favor. At the end this will affect their propagation and the OB will result the most suitable for survival. Indeed, despite the fact that the mutant cells induce a lower proliferation of osteoblasts: this effect is too slight to affect the dynamics in a decisive way. However, if cancer is developed this situation is not plausible. Instead, it is desirable to find it after the effects of a possible cure.

Case $b = 1/2$, $d = 1$

Let us instead assume that, the disadvantage brought to the OB cells, by the presence of the mutant cells is stronger by setting $b + d > 1$. This implies the appearance of another stability point (also belonging to the edge of the simplex) and therefore the prediction on the growth of cancer is more complex.
For this reason, we add the result for a different starting point (Figure 2.4(b)) to show the dependence of the result from the initial conditions: in fact, from two different starting points we reach two different stability results: the defeat of the cancer or the death of all healthy OB cells with serious consequences for the patient.
It should be noted that in this situation a saddle point also appears inside the graph which, precisely, due to the properties of the dynamics, cannot be stable (see appendix).
Furthermore, the time required for convergence (in the case of tumor victory) is very high as one can see in the graph, since the interactions between the OC cells and the mutants are weak.
This particular situation becomes of importance, as we will see below, when the patient undergoes a treatment aiming at lowering the b parameter; in fact, after the cure this one becomes a fairly probable case.

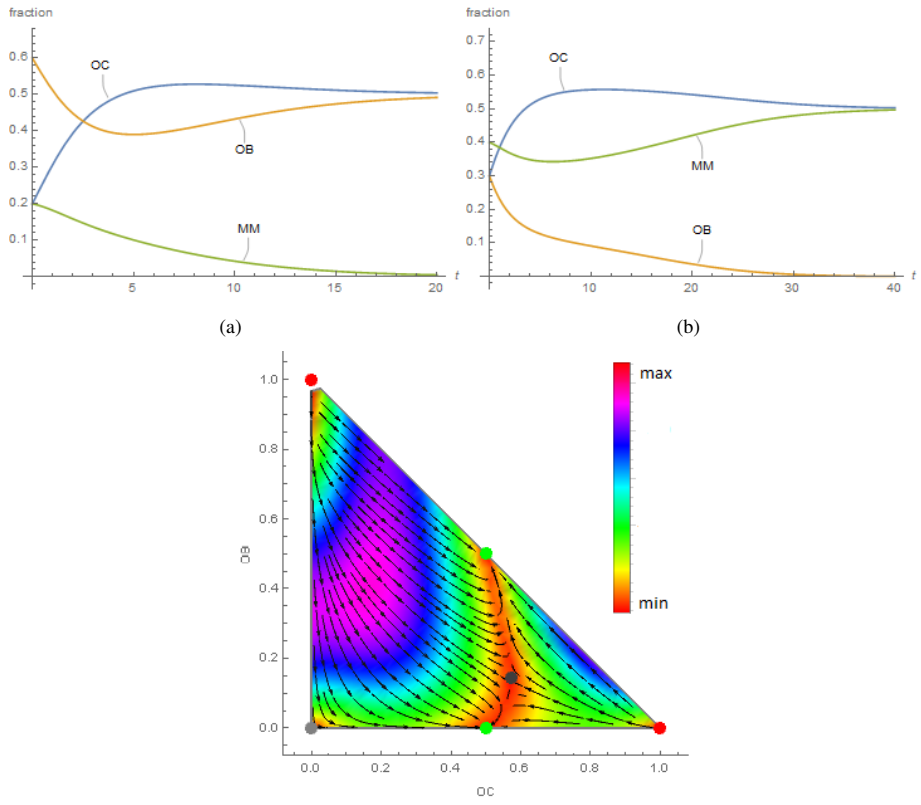
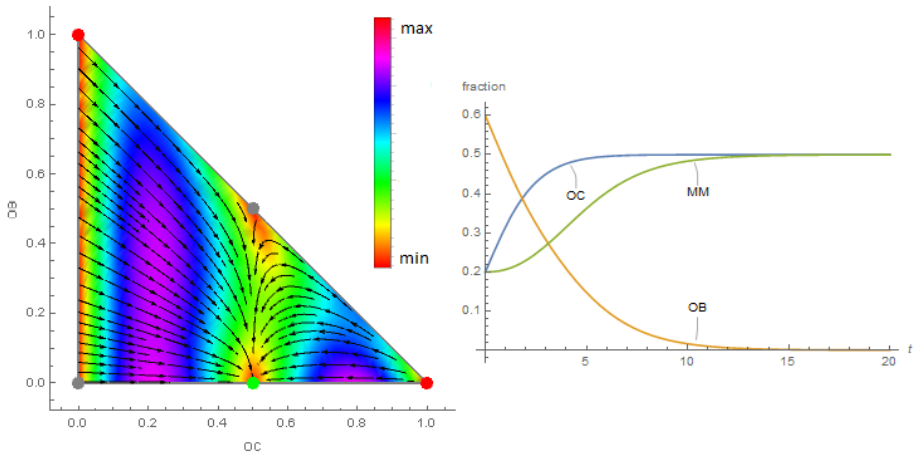


Figure 2.4: $b = 1/2; d = 1$; starting point: $(a) : (x,y,z) = (0.2,0.6,0.2), (b) : (x,y,z) = (0.3,0.3,0.4)$

Case $b = 2$, $d = 0$

Finally, the last and most important situation (Figure 2.5) is the one in which the interaction between OC and mutant cells is very strong. We chose to represent the case in which there is no disadvantage for OB cells since this effect would only affect the convergence speed to the stable equilibrium. This situation still leads to the appearance of a single stable point that corresponds to the coexistence of mutant cells and osteoclasts (OC), and the death of OB cells, with serious consequences.
If the parameter b is greater than 1, which is the value we gave to the interaction between the two groups of healthy cells, the system will evolve in the least desired direction. The situation envisaged by the analytical analysis of the equations is therefore highlighted. In fact, it is straightforward that if the OC cells interact more strongly with the tumor cells than they do with the healthy ones, at the end this behavior will lead to an evolutionary selection in favor of the cancer cells.



(a) (b)

Figure 2.5: $b = 2; d = 0$; starting point: $(x, y, z) = (0.2, 0.6, 0.2)$

With these simple equations we have described the evolution of an ecological system governed by darwinian evolution where species with a better ability to adapt to the surrounding environment are favoured (or in this case to relate to the already present species). Natural selection is the main driver that leads the system towards equilibrium. Few individuals suitable for survival (as in this particular case the MM cells) supplant the inadequate multitude despite their initial minority. A series of mutations, leading to the development of a small group of perfect malignant cells, is all that is needed for cancer to spread, no matter how many they are. Which should be the results if myeloma will be composed of many sub-clones, potentially associated with different clinical behaviour? How would the dynamics evolve due to the new interaction between the several types of clone?

Chapter 3

Sub-clonal populations

3.1 The model

In the three-groups model we consider only one type of mutant cells. This is an approximation since studies [11] indicate that multiple myeloma is characterized by a large number of sub-clonal groups of cells, each type interacting with the other types, and with the healthy cells. Multiple myeloma, thanks to its features, is a perfect pattern in which to study the interaction of several sub-clonal populations and their impact on therapy.

The mutant cells must acquire a complete set of the so-called 'driver' mutations before becoming cancer. In the three-groups model we assume that this event had already been occurred.
However, during the growth of a tumor, mutations called 'passenger' may develop which are not needed for the development of the tumor itself.
Different sub-clonal groups of cells can acquire many types of driver and passenger mutations. These different cancer cells compete for access to limited resources, and the acquisition of 'driver' mutations gives them a survival advantage, leading to clonal dominance.
Studies [11] address the possibility that with targeted treatments against specific sub-clonal populations the disease may become chronic, that is, a situation of coexistence of cancer and health cells may occur, where the cancer, although being incurable, does not lead to the patient's death. In fact, the way the different mutant cells react to therapy depends on the set of driver mutations they have.

We now model the effects of a cure on a dominant sub-clonal population of mutants cells. We introduce a fourth cell population (we will call it MM2, whereas henceforth the mutant cells we previously called MM will be MM1). Whose cells carry a set of driver mutations that further improves (compared to MM) their adaptability and their chances of survival and reproduction. Moreover this is the group of cells on which we assume the treatment is focused. This new population has the same effects as MM1 tumor cells on healthy cells.
Therefore, MM1 and MM2 cells can be seen as two different sub-clonal populations of cancer cells, where the latter group is the dominant one which tries to replace the less adapted one: we can thus schematize their effect on MM1 as a negative interaction.
Furthermore, the cure's effect is modeled by a negative self-interaction parameter for MM2 cells.
To mathematically treat the various cases that can be generated by this new composition of cell groups we write a new payoff matrix $\mathbf{C}$ where $f > 0$ and $h > 0$ are the parameters that regulate the effect of the new MM2 cells on MM1 and MM2, respectively. These parameters come with a minus sign in the matrix because the interactions are assumed to be negative.

$$\mathbf{C} = \begin{array}{c} \\ OC \\ OB \\ MM1 \\ MM2 \end{array} \begin{array}{c} \begin{array}{cccc} OC & OB & MM1 & MM2 \end{array} \\ \begin{pmatrix} 0 & 1 & b & b \\ 1 & 0 & -d & -d \\ b & 0 & 0 & -f \\ b & 0 & 0 & -h \end{pmatrix} \end{array} \tag{3.1}$$

As we have seen before, we can derive the equations that govern the dynamics from the payoff matrix. In what follows x and y are the same as before, z represents the MM1 and w the MM2 :

$$\begin{cases} \dot{x}(t) = x(y + b(z+w) - (2xy + 2bxz - dyz + 2bxw - dyw - fzw - hw^2)) \\ \dot{y}(t) = y(x - d(z+w) - (2xy + 2bxz - dyz + 2bxw - dyw - fzw - hw^2)) \\ \dot{z}(t) = z(bx - fw - (2xy + 2bxz - dyz + 2bxw - dyw - fzw - hw^2) \\ \dot{w}(t) = w(bx - hw - (2xy + 2bxz - dyz + 2bxw - dyw - fzw - hw^2)) \end{cases} \tag{3.2}$$

Following the same procedure as in the case with just three groups of cells, we find the equilibrium points of the system, linearize it, evaluate the Jacobian matrix in such points and finally find its eigenvalues.
This allows us to have information about stability. Below we show the fixed points that we obtained:

$$(x,y,z,w) = (1,0,0,0) \tag{3.3}$$

$$(x,y,z,w) = (0,1,0,0) \tag{3.4}$$

$$(x,y,z,w) = (0,0,1,0) \tag{3.5}$$

$$(x,y,z,w) = (0,0,0,1) \tag{3.6}$$

$$(x,y,z,w) = \left(\frac{1}{2}, \frac{1}{2}, 0, 0\right) \tag{3.7}$$

$$(x,y,z,w) = \left(\frac{1}{2}, 0, \frac{1}{2}, 0\right) \tag{3.8}$$

$$(x,y,z,w) = \left(\frac{d}{1+d+b(-2+b+d)}, \frac{b(-1+b+d)}{1+d+b(-2+b+d)}, \frac{1-b}{1+d+b(-2+b+d)}, 0\right) \tag{3.9}$$

$$(x,y,z,w) = \left(\frac{b+h}{2b+h}, 0, 0, \frac{b}{2b+h}\right) \tag{3.10}$$

$$\begin{pmatrix} x \\ y \\ z \\ w \end{pmatrix} = \begin{pmatrix} \frac{d-h}{1+d+b(-2+b+d)-2h} \\ \frac{b(-1+b+d)-h}{1+d+b(-2+b+d)-2h} \\ 0 \\ \frac{1-b}{1+d+b(-2+b+d)-2h} \end{pmatrix} \tag{3.11}$$

3.2 Stability analysis

Trivial equilibrium

Let us show that the fixed points that present only one type of population (3.3), (3.4), (3.5) and (3.6) are unstable.
Each of these, in fact, possesses at least one eigenvalue with a positive real part, as we can see in Table 3.1.

fixed point (x,y,z,w)	eigenvalues $(\lambda_1,\lambda_2,\lambda_3,\lambda_4)$
$(1,0,0,0)$	$(1,0,b,b)$
$(0,1,0,0)$	$(1,0,0,0)$
$(0,0,1,0)$	$(0,0,b,-d)$
$(0,0,0,1)$	$(h,b+h,-d+h,-f+h)$

Table 3.1: Fixed points and respective eigenvalues

The standard fixed points

We now shift our attention to the fixed points that had already appeared in the three-population model.

The one in (3.7) represents the eradication of cancer and the dynamic balance between the two types of resident cells. One can see that it presents an asymptotic stability only in the case where $b < 1$, like in the previous model. In (3.12) the eigenvalues are shown:

$$(\lambda_1,\lambda_2,\lambda_3,\lambda_4) = \left(-\frac{1}{2},-\frac{1}{2},\frac{1}{2}(-1+b),\frac{1}{2}(-1+b)\right). \tag{3.12}$$

Something different can be seen in the equilibrium in which all OB cells are dead (3.8). In fact, it is no longer possible to assert anything about the stability by linearizing the Jacobian matrix (Lyapunov method), since there is a null eigenvalue.
Only through numerical simulations can we understand its true behavior. However, we can certainly state that it is an unstable equilibrium in the event that $b+d < 1$, since the last eigenvalue would have a positive real part:

$$(\lambda_1,\lambda_2,\lambda_3,\lambda_4) = \left(0,-\frac{b}{2},-\frac{b}{2},\frac{1}{2}(1-b-d)\right). \tag{3.13}$$

The last fixed point we consider in this section is in (3.9), if present, is a saddle point as its correspondent in the three-groups model (2.36).
We report its eigenvalues in the appendix.

The new fixed points

Let us now focus on the study of the two new fixed points that appear when of MM2 cells come to play. The first one in (3.10) is always present and it represents the case of the contemporary death of all OB and MM1 cells, with the consequent victory of the tumor, even if it is composed of MM2 cells.
When studying the stability - i.e. the eigenvalues in (3.14) - we realize that it is asymptotically stable if and only if $f > h$ and, therefore, if the cure's effect on MM2 is less harmful than the effect of MM2 to

MM1 cells.

$$(\lambda_1,\lambda_2,\lambda_3,\lambda_4)=\left(\frac{-b(-1+b+d)+h}{2b+h},\frac{b(-f+h)}{2b+h},-\frac{b^2}{2b+h},-\frac{b(b+h)}{2b+h}\right). \tag{3.14}$$

The last fixed point (3.11) presents a more complex structure. It consists in the simultaneous survival of three different groups of cells.
Since the analytic study of stability depends on many parameters, we resort to numerical simulation to better understand its behavior. We report its eigenvalues in appendix.

3.3 Results

We show some results obtained through simulations, to compare them with those of the previous model. For a better comparison, we use the same initial conditions of the previous model.
The concentration value previously assigned to MM cells is now equally split between MM1 and MM2. We will consider two different cases that depends on the two new parameters.

Case f<h

When $f<h$, treatments have more dangerous effects on MM2 than those that MM2 have on MM1.
In the case $b<1$ and $b+d<1$, we do not expect any substantial difference with respect to three-population case. Indeed, since the tumor was defeated before, the addition of more negative factors -i.e. MM2 cells - may only accelerate its death course. Anyhow, MM2 cells are strongly affected by the effect of the cure that extinguishes them.
Figure 3.1 shows the results obtained by simulation , where we can see the situation converges to the normal homeostasis and the cancer will be eradicated.

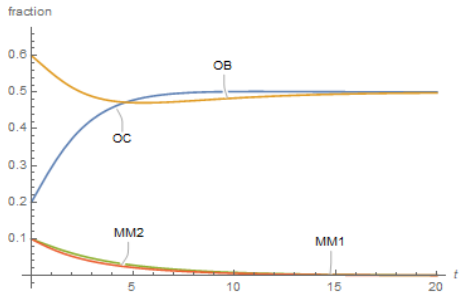
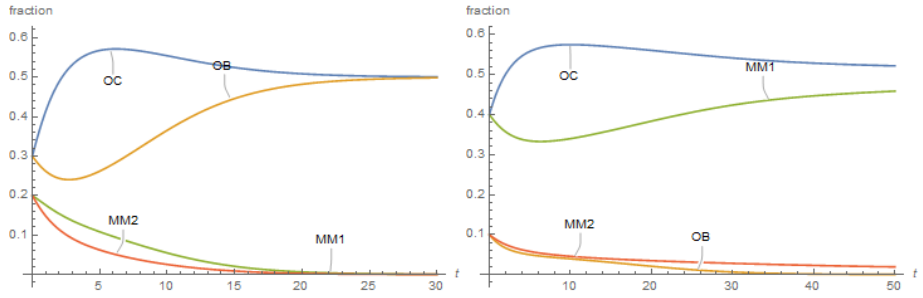


Figure 3.1: $b=1/2; d=1/3; f=1; h=2$; starting point: $(x,y,z,w)=(0.2,0.6,0.1,0.1)$

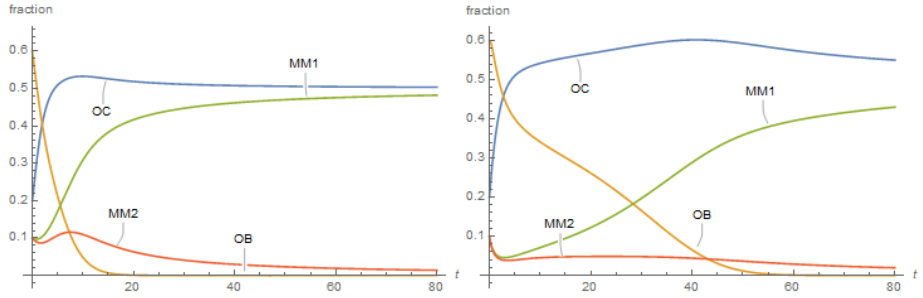
Let us now consider the case $b<1$ and $b+d<1$. Again two different situations may arise depending on the initial concentrations. However, we note that starting with the initial concentration that in the first model would lead to the victory of the tumor (Figure 2.4(b)), now instead leads it to defeat (Figure 3.2(a)). Therefore, more extreme initial conditions (cancer cells are half of the cells present, see Figure 3.2(b)) are needed to fall into the most harmful equilibrium which turns out to be a stable one: this is something that could not be inferred from the analysis of the eigenvalues because one of them is equal to zero, as we can see in (3.13). It also takes much longer to reach it indeed, in the second graphic, convergence has not been reached yet, although we added simulation time. So in this case, it is less unlikely that dynamics evolves towards the victory of the cancer cells.



(a) (b)

Figure 3.2: $b = 1/2; d = 1; f = 1; h = 2$; starting point: $(a) : (x,y,z,w) = (0.3,0.3,0.2,0,2), (b) : (x,y,z) = (0.4,0.1,0.4,0.1)$

The third case presented is the one with $b > 1$ and $d = 0$.
Comparing the two graphs in Figure 3.3, we note that the presence of MM2 cells hampers cancer progression. As a result, the overtaking is slowed down. However, MM2 type cells die nonetheless because of the cure, and cancer eventually overwhelms healthy cells as in the previous model.
The advantage only consists in a longer time of convergence towards the stable equilibrium, therefore allowing to intervening with treatments.



(a) (b)

Figure 3.3: $b = 2; d = 0; (a) : (f,h) = (1,2), (b) : (f,h) = (10,12)$; starting point: $(x,y,z,w) = (0.2,0.6,0.1,0.1)$

In these cases, the only stable equilibria are those found in the three-groups model. Hence, the differences consist in a longer time of convergence to the victory of the tumor and in the value of the initial conditions to be set in order to obtain the same result. Therefore both effects consist of a lower incidence of cancer.

Case f>h

To notice substantial differences between the new and the old model, we must use the condition $f > h$, which implies that the cure's effect against MM2 cells is weaker than damage caused by them to MM1 cells.
The first situation we want to analyze is characterized by $b < 1$. We report three graphs (Figure 3.4) varying d and fixing $f = 2$ and $h = 1$. Figures 3.4(a) and 3.4(b) represent two different cases respectively $d = 1/3$ and $d = 1$, which are the classic situations we are used to analyze (see section 2.4 and Figures 2.4 and 2.5 or in this section Figures 3.1 and 3.2). In both cases the substantial defeat of cancer is

highlighted.
In Figure 3.4(c) we show the result for $d = 10$. This choice leads a great disadvantage for OB cells and the appearance of a different equilibrium where OC cells and MM2 coexist. In this case the cancer survives but at the end it consists of MM2 cells only. This last situation represents the fixed point in (3.10) and underlines its possible stability.

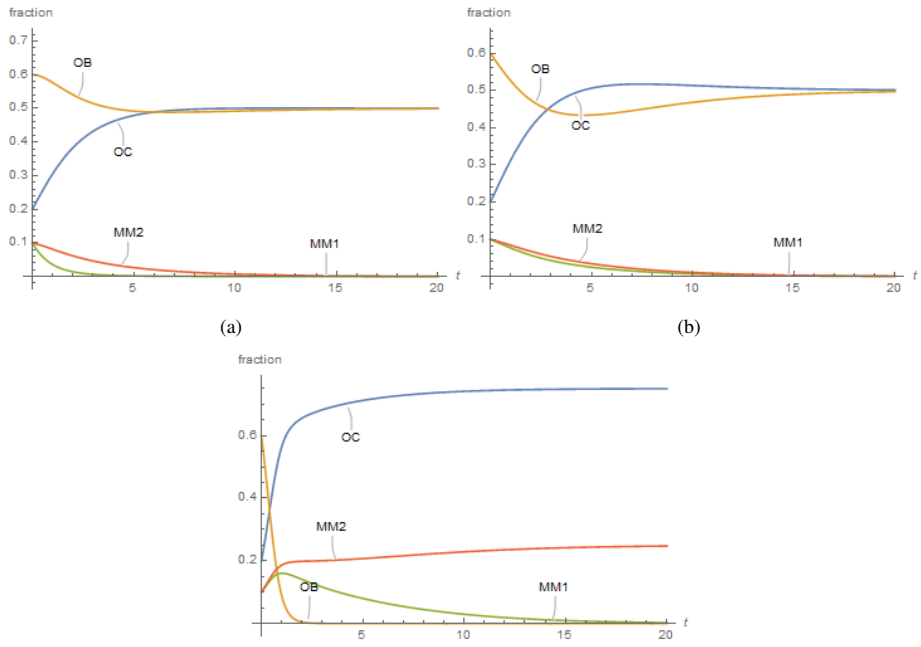


Figure 3.4: $b = 1/2; (a) : d = 1/3, (b) : d = 1, (c) : d = 10; f = 2; h = 1$; starting point: $(x,y,z,w) = (0,2;0,6;0,1;0,1)$

The last case we consider is $b > 1$, that in the three-groups model always led us to the survival of the cancer cells.

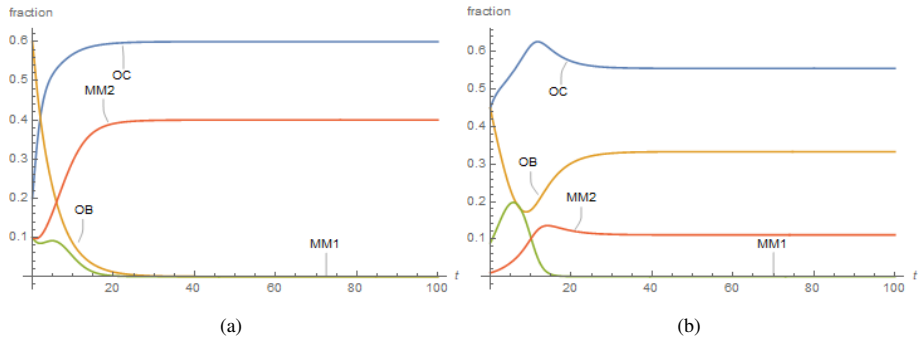


Figure 3.5: $b = 2; d = 0; f = 10; h = 5$; starting point: $(a) : (x,y,z,w) = (0.2,0.6,0.1,0.1), (b) : (x,y,z,w) = (0.45,0.45,0.09,0.01)$

We increase the disadvantage caused by MM2 to try to observe behaviors different from that previously obtained ($f = 10$ and $h = 5$). In Figure 3.5, in fact, we can see that two different situations are

possible, based on the initial frequencies: if the number of MM2 cells is approximately equal to that of MM1, then we fall into the fixed point in (3.10), with the cancer surviving through MM2 cells. On the other hand, if we start with a number of MM1 cells considerably larger than of MM2 cells, a situation of dynamic equilibrium between tumor cells and healthy cells (OB and OC) is created. In this case the MM1 tumor cells go extinct whereas MM2 cells coexist with both types of resident cells. This is fixed point in (3.11). We can thus numerically deduce his possible stability.

After having analyzed in details the several possible cases, we consider now more realistic values for the cell's frequency of $z+w=10^{-10}$ and $OB/OC=1 \rightarrow y/x=1$. Furthermore, we consider a situation in which the tumor can easily develop: $b=3$ and $d=1$.

Regarding the effects that the two different groups of cells have on each other, we can suppose that the effect of cure can be regulated and maintained far below the effect of the interaction between MM1 and MM2 , so we have $f >> h$. The result obtained (Figure 3.6) is of this type (where MM = MM1 + MM2):

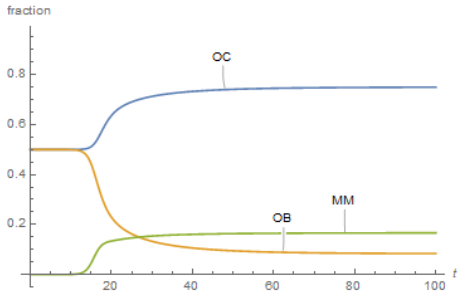


Figure 3.6: $b=3; d=1; f=15; h=10$; starting point: $(x,y,z,w)=(0.5-10^{-10}, 0.5-10^{-10}, 9\cdot 10^{-11}, 1\cdot 10^{-11})$

The system ends up in the last equilibrium point (3.11), in which cells of type OB, OC and MM coexist. This case is what we are looking for. The disease presents a chronic behavior. By attenuating the effect of cure on MM2 the balance is totally in favor of MM2 cells.
In this case the evolutionary thrust brought by the new mutant cells is held back by a group of mutants which possess different characteristics. The MM2 cells become the dominant sub-clone and replace the MM1s. However, their proliferation is kept under control by the specific effect of the treatment, which allows us to maintain cancer in a chronic but non-lethal stage. MM2 cells will grow in number and find stability with the residents. This is an evolutionary stable strategy that corresponds to the contemporary coexistence of the three groups of cells.

Conclusions

Let us summarize what was done. First we relied on the idea of considering a living being as a complex organism, a living ecosystem, and the many cells that compose it as the various species of this ecosystem. This approach allows us to use the methods developed by evolutionary game theory.

So we analyzed a specific part of it, - i.e. the bone -, and we studied the interactions between the main cells within it in the normal condition of homeostasis. Then, we introduced cancer as composed of only one type of mutant cells. From interactions with others, we developed a payoff matrix that represents the rules of the game established between the species - i.e. cells -, we let the replicator equation act on the system, and we obtained results (dependent on only two parameters theoretically unpredictable) on the evolutionary stable strategy in that specific case, and so on the development of the tumor. Finally, we have extended this model to a population of two sub-clones, one of which is better adapted to the surrounding environment than the other. Consequently, let us act a specific therapy to damage it. We analyzed the results obtained under this new, more realistic hypothesis.

Thanks to this three-groups model we can identify some therapeutic indications. In fact, consider the case analyzed in Figure 2.5, a situation in which the tumor has already developed and presents a fair amount of mutant cells: the parameters would lead the dynamics towards cancer victory. It can be verified that through a transplant - which only changes the cell frequencies and leaves the parameters untouched - the dynamics will evolve towards the same equilibrium that would have had previously. Therefore it is essential to find a cure that involves the modification of the parameters that make cancer stronger like b and d.

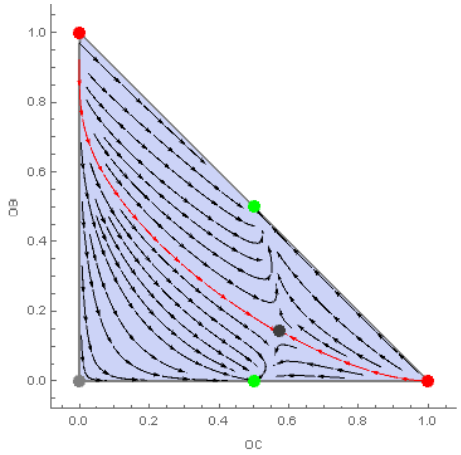


Figure 3.7: $b = 1/2; d = 1$

So, the only way to treat this cancer is to try to lower the parameter b below the critical threshold

value 1. Once this type of treatment has been performed, we find the system in an intermediate situation with $b+d>1$ or $b+d<1$. In the latter case the tumor will be eradicated.
In the first case, on the other hand, there is a saddle point and two points of stable equilibrium. If we want to make the system converge in that which represents normal homeostasis, we can use a transplant that allows us to return to a suitable situation. In fact, the point to which it converges, in this particular case, as we proved before for both proposed models (Figure 2.4, 3.2 and 3.4) for the case $b=1/2$ and $d=1$, depends on the initial conditions. An example of is shown in Figure 3.7. Above the red line there is convergence towards normal homeostasis, below, instead, to the cancer's victory. The behavior depends only on the point of the graph in which we find the system thanks to the cure that lowers b. In case it was below, a transplant would serve to bring the initial condition above the red line. Combined with the cure can therefore save the patient.

The need, therefore, is to know how to derive the correct value of the parameters (and so its situation) for each patient and maximize the success of the chosen treatment.

However, a cure of this kind may clash with the presence of different sub-clonal cancer populations, since the reactions could be different and uncontrollable. Therefore one could try to study the situation using the four-group model which better captures the realistic cancer evolution, although the analysis of the simplest possible case of sub-clonal populations - i.e. only two groups - gives us some important indications: a treatment designed to limit the development of the dominant sub-clone may allow us to regress the disease at a chronic stage (Figure 3.6), suggesting the possibility to successfully treat even diseases previously considered incurable.

The awareness that clonal heterogeneity is significant trait of multiple myeloma has changed our approach to the modelling of cancer, which is now considered as an array of clones and not as a linear evolving disease. Therefore the evolutionary biology becomes of primary importance and also the mathematical methods to study it.

Appendix

Replicator equation and game dynamics

Here, we give a brief mathematical introduction on game theory, the replicator equation and the Lyapunov theorem that uses eigenvalues to determine stability.
These concepts are very important in the study we want to accomplish, as they are the theoretical foundations on which the tools we use are based.
First we will introduce the replicator equation, then taking a look at the game theory we will see how to evaluate the evolution of the various species studied, deducing any convergence to points of stability. Finally we will recall the famous criterion for the equilibrium of dynamic systems.

Derivation of replicator equation

We introduce the main equation we use, that is, the replicator equation. This is the equation with which we describe the temporal evolution of the cell groups. As we shall see, it has properties of invariance, in the sense that the sum of the various populations, if treated by this equation, remains constant over time. This property is particularly useful when dealing with ecosystems with constant resources. However, first let us see how to derive it from pre-existing models [9]. There are two ways we can follow:

From exponential model

The replicator equation can be derived from exponential model: this is the path we will follow because the exponential model is the first elementary we use for our system.
So let us start with the classic exponential growth equation:

$$\dot{N}_i(t) = N_i f_i \qquad i = 1, ..., n \tag{3.15}$$

where N_i is a real function that approximates the population of strategy i and $f_i(N_1, ..., N_n)$ is the fitness of that strategy.
Now we can consider the relative frequencies defined as

$$x_i \equiv \frac{N_i}{P} \tag{3.16}$$

where P is the total population:

$$P(t) = \sum_i N_i(t) \tag{3.17}$$

which obeys to the following differential equation:

$$\dot{P} = \sum_i \dot{N}_i = \sum_i N_i f_i \tag{3.18}$$

Multiplying and dividing (3.18) by P:

$$\dot{P} = P\sum_i \frac{N_i}{P} f_i = P\sum_i x_i F_i = P < F > \tag{3.19}$$

where $< F > \equiv \sum_i x_i f_i$ is the average fitness of whole population.
Now consider (3.16), by product rule and using (3.19) we see:

$$\dot{x_i} = \frac{\dot{N_i}}{P} - \frac{N_i \dot{P}}{P^2} \tag{3.20}$$

$$= \frac{N_i f_i}{P} - \frac{N_i}{P} \underbrace{\frac{\dot{P}}{P}}_{<F>} \tag{3.21}$$

$$= x_i(f_i - < F >) \tag{3.22}$$

Equation (3.22) is for only one specie. Then adding on all the species we obtain

$$\sum_i \dot{x_i} = \sum_i x_i f_i - < F > \sum_i x_i \tag{3.23}$$

That, by the definition of $< F >$, leads to:

$$\sum_i \dot{x_i} = \sum_i x_i f_i - \sum_j x_j f_j \sum_i x_i \tag{3.24}$$

From (3.16) adding on all species:

$$\sum_i x_i = \frac{\sum_i N_i}{P} = \frac{P}{P} \equiv 1 \tag{3.25}$$

Equation (3.24) become using (3.25) the follow identity:

$$\sum_i \dot{x_i} \equiv 0 \tag{3.26}$$

This proves the constancy of total population under a replicator dynamics. As we want to analyze the competition between the several strategies that are included in the population, we discard any environmental effects that could modify the fitness of the strategies. Therefore we can assume that:

$$f_i(N_1, ..., N_n) = F_i\left(\frac{N_1}{P}, ..., \frac{N_n}{P}\right) = F_i(x_1, ..., x_n) \tag{3.27}$$

Using (3.27) the equation (3.22) becomes the well known replicator equation:

$$\dot{x_i} = x_i(F_i - < F >) \tag{3.28}$$

where now

$$< F > = \sum_i x_i F_i \tag{3.29}$$

is completely expressed in terms of the relative frequencies x_i.
Hence, we have obtained the general form of the replicator equation. Mathematically $< F >$ is a term that introduces dependence on the frequencies and fitness of other species. Now let us see how to apply it to an dynamic evolutionary game theory, and study its various properties in this context.

From Lotka-Volterra equations

Hofbauer and Sigmund [4] derived the replicator equation from the Lotka-Volterra equation, also known as prey-predator equations, that are able to describe an ecosystem in which these different species coexist, taking different roles: the n-strategy replicator equation is equivalent to the Lotka-Volterra system with $n-1$ species.
That Lotka-Volterra equation for n population reads

$$\dot{x_i} = x_i \left(r_i + \sum_{j=1}^{n} a_{ij} x_j \right) \qquad i = 1, ..., n$$

where x_i denote the density of i-th species, r_i are the intrinsic growth rates and the a_{ij} model the effect of the j-th upon the i-th population.

Game dynamics

Game theory is the branch of mathematics that studies the choices made by individuals in situations of conflict, where there are other subjects who can make their moves. The decisions undertaken by one can influence those of others, as each individual plays to win, i.e. to maximize total profit. The possibilities are called strategies. Each player receives a pay-off that can be positive, negative or null: it depends on the strategy adopted. We introduce the pay-off matrix, that is the matrix that gives us back the winnings that correspond to a certain interaction. The theory aims to find the equilibrium and study its characteristics.

Evolutionary game theory focuses on the dynamics of strategies. Instead of studying the balance of strategies, it considers their evolution over time. The strategies then become types of populations, that interact through the rules of the game chosen. The payoffs obtained from the game are considered as fitnesses and inserted in the replicator equation, which will produce the new generation that will start playing again.
The replicator equation is the first game dynamics studied in association with evolutionary game theory. It was developed from the biological perspective, in order to predict the evolutionary outcome of population behavior, without a detailed analysis of such biological factors as genetic or population size effects.
The payoffs are translated as the reproductive rate of the species, which are considered large enough to allow each individual to interact with each other (e.g. well-mixed approximation, as it is described in detail in the main part of the text).
The replicator equation tells us how species' frequencies change in time. If the payoff of a certain strategy is constant (independent of strategy frequency), the result obtained shows that each species follows the strategy that maximizes profit, regardless of the equation utilized to describe the dynamic.
In biological terms, according to Darwin's theory of natural selection, we note the survival of the best adapted species.
Lately the theory has achieved successes on the most interesting field, namely the one in which the individual pay-offs depend on the actions perpetuated by the other players. This means that a real dynamic

game is in played. The result that has been proved is that an evolutionary stable strategy (ESS) is stable from the dynamic point of view, if we consider the replicator equation as a regulatory equation of the frequency of the various species.
An evolutionary stable strategy is a behavior that, if adopted by all the resident species in an environment, should be impossible to be invaded by any alternative population that adopt any different strategy. So, evolutionarily speaking, it is a winning strategy which allow resident species to resist the evolutionary pressures due to invasive species, and not to be replaced.

Formally, we start defining an *evolutionary stable type* of behavior of one population [4]:

Definition 3.1 (Evolutionary stable population)**.** If $W(I,Q)$ is the fitness of an individual of type I in a population of composition Q. A population consisting of I-types will be *evolutionary stable* if whenever a small amount of deviant J-types is introduced, the old type I fares better than the newcomers J. So for all $J \neq I$,

$$W(J, \varepsilon J + (1-\varepsilon)I) < W(I, \varepsilon J + (1-\varepsilon)I)$$

for all sufficiently small $\varepsilon > 0$.

We now need an important concept of game theory: the Nash equilibrium (NE). If each player does not benefit from a unilateral change of strategy, then it prefers to keep the current one: this is the fundamental idea hides behind this notion of equilibrium. This is a strategy with the best reply against itself. It may not correspond to the best strategy ever, however there are no reason for a single player to change it, since he would end up in a more disadvantageous situation.
Consider a game with two players only, who play two different strategies $\mathbf{p}$ e $\mathbf{q}$; a strategy corresponds to a point in the simplex:

$$S_N := \left\{ \mathbf{p} = (p_1, ..., p_N) \in \mathbb{R}^n : \sum_{i=1}^{n} p_i = 1, \quad p_i \geq 1, for \ \ i = 1..n \right\}$$

and $\mathbf{U} = u_{ij}$ is a NxN matrix. Therefore this is a matrix game where u_{ij} is the payoff to i when playing against j. So in this case we are assuming that the function F_i is linear and hence, it can be rewritten in terms of the payoff matrix $\mathbf{U}$:

$$F_i = (\mathbf{Uq})_i = \sum_j u_{ij} q_j$$

We formally define a *Nash equilibrium*:

Definition 3.2 (Nash equilibrium)**.** A strategy $\mathbf{q}$ is define a *Nash equilibrium* if

$$\mathbf{p} \cdot \mathbf{Uq} \leq \mathbf{q} \cdot \mathbf{Uq}$$

hold for all strategies $\mathbf{p} \neq \mathbf{q}$ in S_N.

The meaning of this definition is: if a player follows the pre-established rules of the game, described by the matrix $\mathbf{U}$, and he uses his strategy, which turns out to be of Nash's equilibrium, then, no other strategy can guarantee him a higher pay-off. If now we consider two players who use the same *strict* Nash equilibrium strategy $\mathbf{q}$, every individual deviating from it will be penalized.

Now we give the statement of the Folk theorem without prove it [6]. It involves the definition of equilibrium of Nash that we have just enunciated and its evolution under the replicator equation, where the strategy become the population.

Theorem 3.3.1 (Folk Theorem)**.**
The replicator equation for a matrix game satisfies:

1. *A stable rest point is a NE;*
2. *A convergent trajectory in the interior of the strategy space evolves to a NE;*
3. *A strict NE is locally asymptotically stable.*

This theorem allows us to predict the evolutionary outcome of stable ecological systems by examining NE behavior of the game.
Joining the two definitions given before, the two types J and I become the strategy $\mathbf{p}$ and $\mathbf{q}$, so we can say that [4]:

Definition 3.3 (Evolutionary stable strategy (ESS))**.** The strategy $\mathbf{q}$ is an *evolutionary stable strategy (ESS)* if for all $\mathbf{p}$ with $\mathbf{p} \neq \mathbf{q}$ the inequality:

$$\mathbf{p} \cdot \mathbf{U}(\varepsilon\mathbf{p} + (1-\varepsilon)\mathbf{q}) < \mathbf{q} \cdot \mathbf{U}(\varepsilon\mathbf{p} + (1-\varepsilon)\mathbf{q})$$

for all sufficiently small $\varepsilon > 0$ i.e. smaller than an appropriate invasion barrier $\hat{\varepsilon}(\mathbf{p}) > 0$ that is, the maximum number of mutants against which the resident species can resist [7].

Manipulating this equation we obtain:

$$(1-\varepsilon)(\mathbf{q} \cdot \mathbf{U}\mathbf{q} - \mathbf{p} \cdot \mathbf{U}\mathbf{q}) + \varepsilon(\mathbf{q} \cdot \mathbf{U}\mathbf{p} - \mathbf{p} \cdot \mathbf{U}\mathbf{p}) > 0$$

So $\mathbf{q}$ is a evolutionary stable strategy if and only if it satisfies these two conditions:

1. *equilibrium condition (NE condition):*

   $$\boldsymbol{q} \cdot \boldsymbol{U}\boldsymbol{q} \geq \boldsymbol{p} \cdot \boldsymbol{U}\boldsymbol{q}$$

   for all $\boldsymbol{p} \in S_N$

2. *stability condition:*

   $$\boldsymbol{q} \cdot \boldsymbol{U}\boldsymbol{p} > \boldsymbol{p} \cdot \boldsymbol{U}\boldsymbol{p}$$

   if

   $$\boldsymbol{p} \neq \boldsymbol{q} \quad \wedge \quad \boldsymbol{q} \cdot \boldsymbol{U}\boldsymbol{q} = \boldsymbol{p} \cdot \boldsymbol{U}\boldsymbol{q}$$

   for all $\boldsymbol{p} \in S_N$

The first condition is nothing other than the definition of a Nash equilibrium, i.e. that the strategy which is the best reply against itself. However, this condition does not guarantee that there cannot be another strategy that is an alternative best reply. And here comes the second condition that guarantees local uniqueness: it assures us that in the case of equality between the two strategies, the $\mathbf{q}$ returns a greater value against $\mathbf{p}$ than what $\mathbf{p}$ returns against itself.
That is strict NE implies ESS and vice versa.

Now we can enunciate a theorem [6] which presents us with some relevant properties of the ESS in association with the replicator equation. The last two points are a recent result of the evolutionary game theory:

Theorem 3.3.2.

1. *The strategy* $\boldsymbol{q}$ *is an ESS if and only if*

$$\boldsymbol{q} \cdot \boldsymbol{U}\boldsymbol{p} > \boldsymbol{p} \cdot \boldsymbol{U}\boldsymbol{p}$$

   for all $\boldsymbol{p} \neq \boldsymbol{q}$ *sufficiently close (but not equal) to* $\boldsymbol{q}$ *in* S_N*;*

2. *An ESS* $\boldsymbol{q}$ *is a locally asymptotically stable rest point of the replicator equation;*

3. *An ESS* $\boldsymbol{q}$ *in the interior of* S_N *is a globally asymptotically stable rest point of the replicator equation.*

Actually the two lasts statements are very important: if $\mathbf{q} \in int(S_N)$ is an evolutionary stable strategy, then there cannot be other *ESS*. Hence, there will be games in which there is none, or games with many *ESS* that, however, have to lie in the boundary of S_N.

We succeeded in combining a concept of ecological population dynamics as an evolutionary stable type of population, with a fundamental one of game theory such as the Nash equilibrium. The dynamics of the populations has been merged to the static nature of the strategies, and from this union the definition of ESS was born.
So by a simply study regarding the stability properties of the replicator equation, we can infer information about the adopted evolutionary strategy, and this is what we are going to do in the models.

Stability of equilibria

Therefore, once the differential equation system associated with the problem is obtained, we will linearize it to obtain information on the stability of equilibria.
So, the first Lyapunov theorem becomes of fundamental importance [5]:

Theorem 3.3.3 (First Lyapunov theorem)**.**
Suppose that z^* *is an equilibrium of the equation* $\dot{z} = X(z)$ *with* $z \in \mathbb{R}^n$.

1. *If* $\frac{\partial X(z)}{\partial z}(z^*)$ *has all the eigenvalues with negative real part, then* $z*$ *is asymptotically stable;*

2. *If* $\frac{\partial X(z)}{\partial z}(z^*)$ *has at least one eigenvalue with positive real part, then* z^* *is unstable.*

It teaches us that in the study of equilibrium stability, each eigenvalue of the Jacobian matrix must have a strictly negative real part in order to have asymptotic stability. Instead it is enough to have only one eigenvalue with a positive real part to have instability. It therefore provides both conditions necessary for asymptotic stability and sufficient for instability.
However, the Lyapunov method gives no information on the nonlinerized system when a stable equilibrium of the linearized one has at least one null eigenvalue (not asymptotically stable).
Hence, only thanks to numerical simulations we can verified the actual stability of a fixed point.

Stability analysis for the equilibria of the 3-species cell model

Here we study the stability of the equilibria for system (2.28). The Jacobian of the system reads:

$$J(x,y,z)=\begin{pmatrix} y-4xy+bz-4bxz+dyz & x(1-2x+dz) & x(b-2bx+dy) \\ y(1-2y-2bz) & d(-1+2y)z+x(1-4y-2bz) & y(-d-2bx+dy) \\ z(b-2y-2bz) & z(-2x+dz) & bx-2xy-4bxz+2dyz \end{pmatrix}$$

The equilibrium $(x,y,z)=(1,0,0)$ results to be unstable as the Jacobian has two positive eigenvalues. It results

$$J(1,0,0)=\begin{pmatrix} 0 & -1 & -b \\ 0 & 1 & 0 \\ 0 & 0 & b \end{pmatrix} \quad \text{and} \quad (\lambda_1,\lambda_2,\lambda_3)=(1,0,b).$$

The Jacobian matrix evaluated at $(x,y,z)=(0,1,0)$ has only one eigenvalue, which is positive, different from zero. This implies the equilibrium is unstable.

$$J(0,1,0)=\begin{pmatrix} 1 & 0 & 0 \\ -1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \quad \text{and} \quad (\lambda_1,\lambda_2,\lambda_3)=(1,0,0).$$

The equilibrium $(x,y,z)=(0,0,1)$ is a saddle point as the non-zero eigenvalues have opposite sign.

$$J(0,0,1)=\begin{pmatrix} b & 0 & 0 \\ 0 & -d & 0 \\ -b & d & 0 \end{pmatrix} \quad \text{and} \quad (\lambda_1,\lambda_2,\lambda_3)=(0,b,-d).$$

We evaluate the Jacobian matrix in $(x,y,z)=\left(\frac{1}{2},\frac{1}{2},0\right)$

$$J(\tfrac{1}{2},\tfrac{1}{2},0)=\begin{pmatrix} -\frac{1}{2} & 0 & \frac{d}{4} \\ 0 & -\frac{1}{2} & \frac{1}{2}\left(-b-\frac{d}{2}\right) \\ 0 & 0 & -\frac{1}{2}+\frac{b}{2} \end{pmatrix} \quad \text{and} \quad (\lambda_1,\lambda_2,\lambda_3)=\left(-\tfrac{1}{2},-\tfrac{1}{2},\tfrac{1}{2}(-1+b)\right).$$

The Jacobian matrix in $(x,y,z)=\left(\frac{1}{2},0,\frac{1}{2}\right)$ reads:

$$J\left(\tfrac{1}{2},0,\tfrac{1}{2}\right)=\begin{pmatrix} -\frac{b}{2} & \frac{d}{4} & 0 \\ 0 & \frac{1}{2}-\frac{b}{2}-\frac{d}{2} & 0 \\ 0 & \frac{1}{2}\left(-1+\frac{d}{2}\right) & -\frac{b}{2} \end{pmatrix} \quad \text{and} \quad (\lambda_1,\lambda_2,\lambda_3)=\left(-\tfrac{b}{2},-\tfrac{b}{2},\tfrac{1}{2}(1-b-d)\right).$$

The analysis of the sign of these eigenvalues is reported in the section 2.3, where for each case we deduced the properties changing the values of the parameters

Checking now in the last equilibrium:$(\bar{x},\bar{y},\bar{z})=\left(\frac{d}{1-2b+b^2+d+bd},\frac{b(-1+b+d)}{1-2b+b^2+d+bd},\frac{1-b}{1-2b+b^2+d+bd}\right)$:

$$J(\bar{x},\bar{y},\bar{z})=\begin{pmatrix} -\frac{2bd^2}{(1+d+b(-2+b+d))^2} & \frac{(-1+b)^2d}{(1+d+b(-2+b+d))^2} & \frac{bd(-1+b+d)^2}{(1+d+b(-2+b+d))^2} \\ \frac{(-1+b)b(-1+b-d)(-1+b+d)}{(1+d+b(-2+b+d))^2} & -\frac{b(1+b)d(-1+b+d)}{(1+d+b(-2+b+d))^2} & -\frac{bd(-1+b+d)(1+b+d)}{(1+d+b(-2+b+d))^2} \\ -\frac{(-1+b)^2b(-1+b+d)}{(1+d+b(-2+b+d))^2} & \frac{\left(-1+b^2\right)d}{(1+d+b(-2+b+d))^2} & -\frac{(-1+b)bd(-3+b+d)}{(1+d+b(-2+b+d))^2} \end{pmatrix}$$

We find the eigenvalues:

$$\begin{pmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \end{pmatrix}=\begin{pmatrix} \frac{bd}{1+d+b(-2+b+d)} \\ -\frac{bd(1+d+b(-2+b+d))+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)(1+d+b(-2+b+d))^2}}{2(1+d+b(-2+b+d))^2} \\ \frac{-bd(1+d+b(-2+b+d))+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)(1+d+b(-2+b+d))^2}}{2(1+d+b(-2+b+d))^2} \end{pmatrix}$$

These eigenvalues can be simplified considering the condition $\frac{d}{1-2b+b^2+d+bd}\geq 0$ found previously during the existence tests.

$$\left(\frac{bd}{1+d+b(-2+b+d)},-\frac{bd+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)}}{2(1+d+b(-2+b+d))},\frac{-bd+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)}}{2(1+d+b(-2+b+d))}\right)$$

Here we reported only the simplify eigenvalues, in fact the stability of the latter is investigated in section 2.3 because arguments concerning the existence of equilibrium intervene.

Stability analysis for the equilibria of the 4-species cell model

For the four-group model, we do not report the steps that are the same as the previous one. Instead we show the eigenvalues of the Jacobian matrix evaluated in the fixed point which have appeared following the addition of the new cell type. Relying on the system (3.2), we derived and evaluated the Jacobian matrix in the equilibrium points. We focus only on those that present significant differences with the previous model: The eigenvalues of the first equilibrium $(x,y,z) = \left(\frac{b+h}{2b+h}, 0, 0, \frac{b}{2b+h}\right)$ are:

$$\begin{pmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \\ \lambda_4 \end{pmatrix} = \begin{pmatrix} 0 \\ -\frac{bd}{1+d+b(-2+b+d)} \\ \frac{bd(1+d+b(-2+b+d))+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)(1+d+b(-2+b+d))^2}}{2(1+d+b(-2+b+d))^2} \\ \frac{-bd(1+d+b(-2+b+d))+\sqrt{-bd\left(4(-1+b)^2+(-4+3b)d\right)(1+d+b(-2+b+d))^2}}{2(1+d+b(-2+b+d))^2} \end{pmatrix}$$

the eigenvalues of the second one $(x,y,z) = \left(\frac{d-h}{1+d+b(-2+b+d)-2h}, \frac{b(-1+b+d)-h}{1+d+b(-2+b+d)-2h}, 0, \frac{1-b}{1+d+b(-2+b+d)-2h}\right)$ are $(\lambda_1, \lambda_2, \lambda_3, \lambda_4)$:

$$\begin{pmatrix} \frac{(-1+b)(f-h)}{1+d+b(-2+b+d)-2h} \\ \frac{-bd+h}{1+d+b(-2+b+d)-2h} \\ -\frac{b(1+d+b(-2+b+d)-2h)(d-h)+\sqrt{-(1+d+b(-2+b+d)-2h)^2(d-h)\left(4(-1+b)^2b+b(-4+3b)d+(-2+b)^2h\right)}}{2(1+d+b(-2+b+d)-2h)^2} \\ \frac{-b(1+d+b(-2+b+d)-2h)(d-h)+\sqrt{-(1+d+b(-2+b+d)-2h)^2(d-h)\left(4(-1+b)^2b+b(-4+3b)d+(-2+b)^2h\right)}}{2(1+d+b(-2+b+d)-2h)^2} \end{pmatrix}$$

as we can see, these two groups of eigenvalues present a complex form that affects the analytic study. In fact the nature can be more easily understood relying on a numerical analysis of the solution.

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