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Cancer differentiation therapy dynamics in a hybrid model of avascular tumor growth

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Summary/Abstract

Cancer is not one disease but many, making it very difficult to successfully develop treatments that effectively end it. Yet, a very promising approach still underdeveloped is differentiation therapy (DTH). As it has long been demonstrated, most tumors are conformed by a cell population a great part of which is poorly differentiated, exhibiting a loss of communication and tissue homeostasis among other things. As a result, they can achieve several hallmarks essential to their identity and chances of success. DTH has been proposed to be an efficient therapy and can be combined with cytotoxic-based therapies, and successfully used as a treatment for acute promyelocytic leukemia (APL). However, DTH has failed so far when dealing with solid tumors. Why? It has been suggested that the high degree of spatial intra-tumoral heterogeneity combined with the resilience of CSCs and their capability to repopulate tumors by themselves might act as a firewall to DTH drugs. In order to test this possibility and assess the effects of DTH on solid tumors, we propose a mathematical and computational study of DTH using a spatially extended avascular tumor model. The results obtained support the previous hypothesis and indirect evidence concerning the role of space and cancer tissue architecture.

Keywords

Cancer, differentiation therapy, competition, ecology, habitat fragmentation, cancer stem cells, complex systems

Preface

Because of its intrinsic complexity, a systems-level understanding of biological systems often requires the use of mathematical and computational tools beyond the traditional methods of biological sciences. The success of these methods is illustrated by the rise of a new field, Systems Biology, that has been percolating multiple domains of the biological sciences over the last two decades, from molecular and cell biology to complex diseases.

Many scientific advances, particularly within biomedical research, take place nowadays at the boundaries between disciplines. Old problems are being now reformulated in novel ways by taking advantage of fresh ways of looking at living matter from mathematics, physics or engineering perspectives. Many different areas exemplify how unique and useful interdisciplinarity is, and one of them is the Scientific modelling. This area aims at making a particular feature of the world easier to understand, simulate and analyze, by properly formulating models of a given complex biosystem. Furthermore, an important part of this field is the study of diseases, some of which are too complex to be captured in simple linear metaphors. In many cases, models are becoming our bridge between disease and potential treatments. In this context, the role of biomedical engineers proves to be key thanks to the capability to combine the modelling of systems with the biomedical research, which can be put to good use in the study of diseases, sometimes thinking out of the box. It is in this aspect in which I wish to contribute with my research.

The first step towards that contribution has been the development of a novel computational model that provides a deeper insight into the possible use of so called differentiation therapy for the treatment of solid tumors, and what are its main strengths and weaknesses. Differentiation therapy, or DTH, has already proven its effectiveness in liquid tumors, due to their low degree of heterogeneity amongst other things, but its use against solid tumors has proven to be a challenge that needs to be thoroughly studied, since many unknowns and questions remain open and need to be addressed.

Hence, the objective of this work is to analyze the behavior of a spatially-extended cancer cell population that is under a simulated DTH treatment, identifying key points of action during the simulated therapy, as well as the potential weaknesses related to the ecology of the tumor that can be exploited to achieve a successful outcome, which is an approach that not many studies have taken so far. Furthermore, the key role of cancer stem cells (CSCs) is also considered and focused upon. In a nutshell, the question posed here is: why DTH might have failed so far to work in solid tumors and what would be needed to overcome its limitations?

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1 Introduction

One of the main diseases that overshadow our world nowadays is cancer, which is not a single disease but rather a set conformed by multiple disorders. Its success comes as a direct effect of Darwinian evolution (*Greaves & Maley, 2012*). Such disorder comes from mutations that can happen in a single somatic cell, that turns into a completely new one, the proliferative capability of which is increased, and can trigger a series of events that include formation of a tumor and metastases. That is due to the fact that, according to the clonal origin of cancer (*Fialkow, 1976; Nowell, 1976; Sidransky et al., 1992; Wang et al, 2009*), a single mutation in one cell can therefore give place to the development of an entire tumor. Nevertheless, random mutations can take place in it, and new subpopulations of the tumor will originate. Considering the fact that they live in a limited space with limited resources, they will start competing, and following the Darwinian laws, only the fittest will survive. Such populations capable of surviving will eventually elude the surveillance present in the human body as exemplified in Figure (1), being able to survive and thrive in an otherwise hostile environment for them. In the process of development, cancer cells reach 'Hallmarks' (*Hanahan & Weinberg, 2011*) such as enabling immortality, which provide them with the tools necessary to go from a normal cell to a hostile machine capable of taking over their habitat and extending to other sites of the host, eventually dominating their counterpart.

Reaching the aforementioned hallmarks serves as the starting point for the development of cancer's deadliest trait: its metastatic action, which is the precursor of tumor recurrence as seen in Figure (1). Studies suggest that metastases is responsible for about 90% of all cancer deaths (*Guan, 2015*). Such critical statistic allows for the understanding of how dangerous the aforementioned process is during the development of cancer. Nevertheless, there are different threats that cells from the primary tumor must overcome in order to successfully metastasize. Some examples that allow for a better understanding of this process are the invasion of adjacent tissues, intravasation, transport of cancer cells through the circulatory system and extravasation and growth in a secondary organ (*Mazzocca & Carloni, 2009*). Each of this steps has a high degree of complexity, and that is the main reason why though they would be ideal therapeutic targets, the lack of knowledge related to their molecular functioning makes most therapies inefficient.

There is a number of classical, well-established therapies (and combinations of them) that are being employed nowadays to fight cancer back. The most standard treatments being employed are surgery, chemotherapy or radiotherapy. Surgery is a common approach taken in many treatments, and it consists in the removal of cancer from the body and some of its nearby tissue. Nevertheless, pain and infections as well as cancer relapse are very common side effects. The use of cytotoxic drugs in order to kill fast-growing cells is another of the most remarkable approaches, called chemotherapy, and is usually employed as a resort in the latter stages of development of the disease. Furthermore, this technique can also be combined with radiotherapy, which consists in the use of radiation to kill cancer cells and shrink tumors. Though

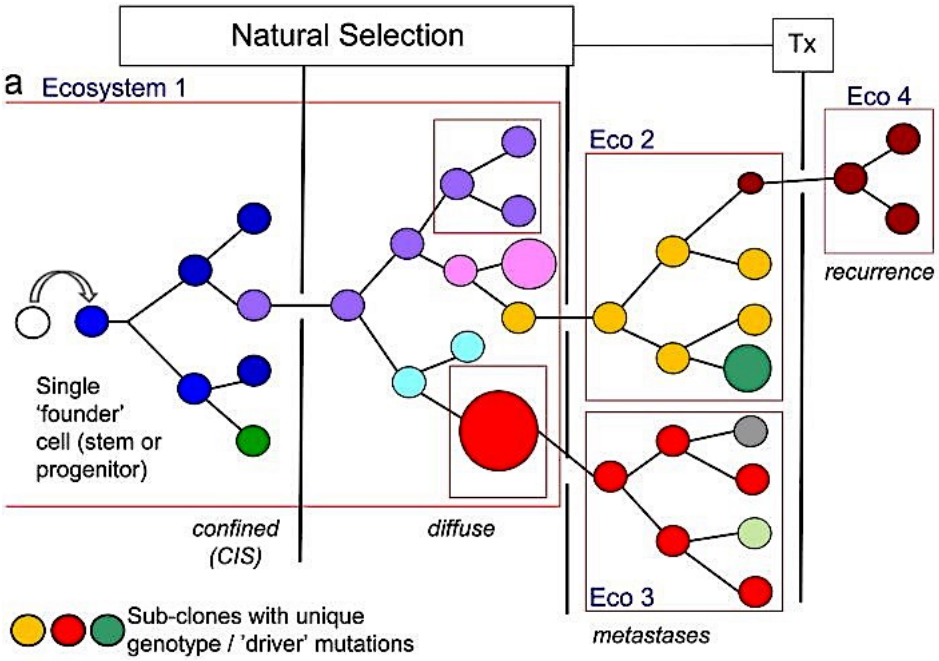

Figure 1: Branching clonal evolution of cancer, where selective pressures play a key role. Following the clonal evolution of cancer, and with the acquisition of driver mutations, successful waves of tumor expansion and the eventual development of metastases is bound to take place. *Greaves & Maley, 2012*

one of the most used, current studies aim at reducing radiation therapy related toxicities, which is a common problem that arises (*Baskar et al, 2012*). Furthermore, targeted therapies are also seeing its use increase as of lately, since targeting specific pathogenic pathways has proven to be an effective approach, and an example of it is immunotherapy, which is a therapy that works by either activating or suppressing the immune system. Plenty of different approaches can be taken, such as the administration of BCG vaccine for bladder cancer or administration of monoclonal antibodies, used in different types of solid tumors among others. Other treatments include hormone therapies and apoptosis inducers.

As can be extracted from the previous explanation, the mechanism of action of both chemotherapy and radiotherapy is cell damage and, as a result, cell death (*Bhosle & Hall, 2009*). Nonetheless, this *modus operandi* triggers an undesired evolutionary pressure on the cancerous cells. When being placed under survival-threatening conditions, selective pressure will be placed upon the tumoral population so that resistant cells can proliferate. Such cells will be characterized by the capability of resisting therapeutic treatments, and the mechanism by which they will do it can be very different (*Greaves & Maley, 2012*). Those clones will contain 'driver' mutations, that enable cancer cells to reach their objective.

In this context, there is a need to study cancer from an evolutionary and ecological standpoint to find alternatives to fight cancer resistance back. The approach of evolutionary biology in this field has long been overlooked (*Merlo et al., 2006*).

Nonetheless, this is starting to change (*Gonzalez-García et al., 2002; Maley et al., 2004; Maley et al., 2006*). As explained before, the main treatments employed lack effectiveness, since the selective pressures mentioned above cause undesirable side effects, being the trigger of metastases one of them. Hence, an ecological approach can help shape a better strategy to fight back cancer, since cancer is affected by the interactions present between the individuals, in this case cancerous cells, and their environment (*Basanta & Anderson, 2013*). This view is shared by any other ecosystem of our world.

Some classic experiments paved the way to understand the role of competition and mutation in evolving populations. For instance, an experiment (*Luria & Delbrück, 1943*) done with the purpose of understanding how a bacterial culture might be affected by the presence of a bacterial virus, or phage, showed that the exposure of such culture to an aggressive threat posed selective pressure towards that population, eventually resulting in selection of pre-existing mutants. Hence, this behavior might take place in cancer as well, although research has shown that intratumor heterogeneity and resistance to therapies comes as a combination of many different factors, meaning that this problem is far more multi-dimensional (*Sottoriva et al., 2013*). Furthermore, that intratumor heterogeneity is closely tied to the presence of cancer stem cells (CSCs), the asymmetrical division of which leads to the development of such heterogeneous landscape (*Rich,2007; Lathia et al., 2011; Bao et al., 2013*).

Taking into account this principle, time can be expected to play a key role in the capability of cancer to produce resistant mutants to therapies. Tumors need a number of mutations before creating a neoplasm aggressive enough. Tumors such as the ones that appear in the brain and nervous system, endocrine and the eye require less mutations than others such as the ones appearing in the prostate and larynx (*Balmain et al., 1993*). That is the main reason why early detection of cancer before the appearance of intertumoral wide-spread heterogeneity reduces mortality, as well as morbidity and costs (*Etzioni et al., 2003*).

Within this framework, there is one interesting and promising alternative to the traditional approach taken to fight cancer that can take into account many of the premises previously mentioned, and that is differentiation therapy. One of the main traits of cancer cells is their ability to evade or block differentiation, which results in cell with greater potential to generate more cell types, as well as maintaining their ability to self-renew. That is due to the fact that, as a cell continues differentiating, its potency decreases, since the differentiation of a cell can be seen as the terminal point of its growth, where all potency is lost (*Warren et al., 2010*). Notice also the morphological change that cells that have undergone differentiation experience in Figure (2).

As a result, a completely differentiated cell has the minimal possible potency, with only having one task as its purpose. Hence, cancer cells look for the possibility of having a higher potency, including potential to self-renew as well as maintaining

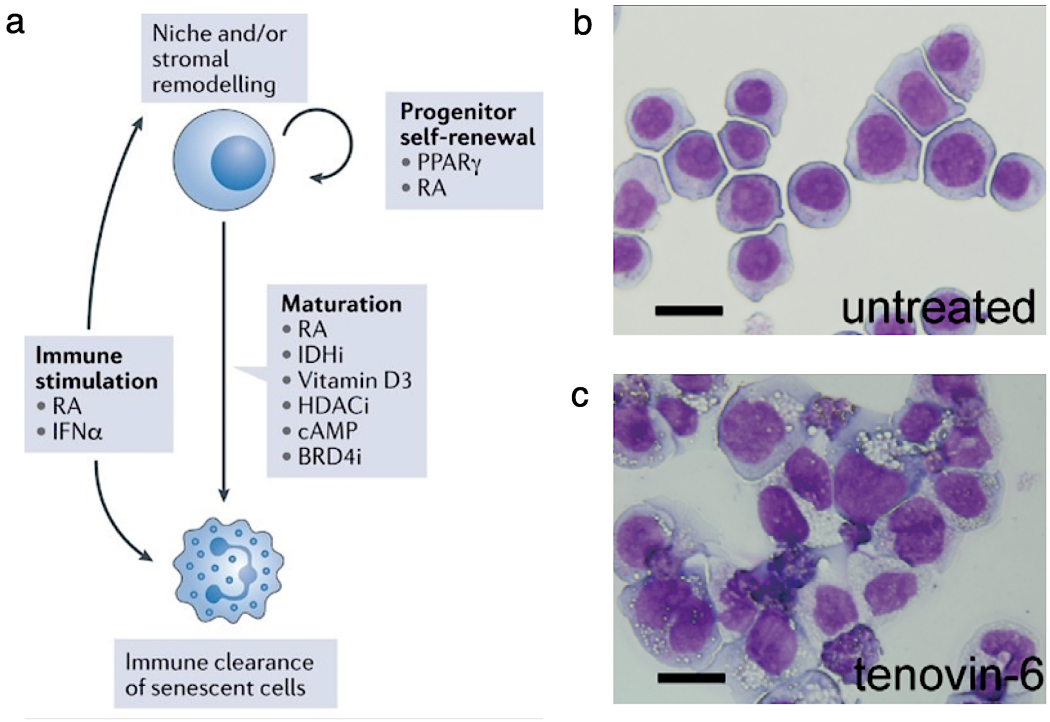

Figure 2: (a) Transitions from cancer to differentiated cells. This is a process in which different mutagenic agents may play a key role moving forward. Extracted from *de Thé, 2018*. (b) Two examples of leukemia cells (b) untreated and (c) treated with DTH. Notice the shift from the round, featureless shape to the characteristic terminal differentiation phenotype.

a high rate of proliferation. As a result, a very important hallmark as sustaining proliferative signaling can be reached. Taking this into account, differentiation therapy can be employed so that those cancer cells lose their potential as a result of a certain drug or agent (*Spira & Carducci, 2003*).

This therapy was suggested for the first time 50 years ago (*Pierce & Wallace, 1971*). They studied the effect of using a given agent to differentiate undifferentiated cancer cells, and they analyzed the development of the tumor, eventually noticing that differentiated cells were unable to form a tumor by themselves. As a result, not only the potency of undifferentiated cancer cells was understood, but also the possibility of a transition from malignant, undifferentiated cells to benign differentiated cells was first achieved. Hence, such therapy is based on one of the main hallmarks of cancer, which is the block or loss of differentiation, leading to undifferentiated, highly potent cancer cells.

As it was also pointed out by Stuart Kauffman (*Kauffman, 1971*), cells in the organism are affected by chemical fluctuations, which cause the system to pass through its different modes of stable behavior. Nonetheless, several experiments and models (*Hadorn, 1966; Rivera & Bennett, 2010*) have shown that the organism uses only a determined and small subsets of cell types that have no mutations, which means

that there are plenty of subsets with mutations introducing the idea that epigenetic cancer is, at the very least, possible. Among those mutations, different differentiation pathways may be affected (*Jones & Baylin, 2002*), inducing the development of populations that follow this same mutations, subsequently developing a culture of undifferentiated cells. As he also notes, this would mean that this neoplasia can be differentiated, turning its nature into non-malignant behavior, indicating that the basis upon which differentiation therapy works have been theorized for a long time.

This opens up a new spectrum of possibilities, since we could induce differentiation in cancer cells, turning previous malignant cells into benign cells by virtue of molecular mutagenic agents. This is the part where differentiation therapy can play a big role moving forward. Though still in its early stages of development, especially in solid tumors (*Yan & Liu, 2016*), there have been studies that have already proven the effectiveness of employing DTH to treat some kinds of tumors. Retinoic acid in particular has been demonstrated to be a successful treatment for nasopharyngeal carcinoma (*Yan et al., 2014*), as well as yielding efficient results for treating acute promyelocytic leukemia (*Tallman et al., 2002*). Furthermore, the use of such agent against neuroblastoma in children has also delivered promising results (*Matthay et al., 2009*). Nevertheless, this promising results are the beginning of what figures to be a long road of study, since matters such as inter-tumor and inter-patient variability among others pose a hurdle towards the successful development of this new branch of treatments (*Yan & Liu, 2016*).

Differentiation therapy was first successfully employed as treatment for acute promyelocytic leukemia (APL) which is a subtype of acute myeloid leukemia characterized by a specific abnormality, involving chromosomes 15 and 17 (*Welch et al., 2012*). More specifically, it involves a translocation that affects the retinoic acid-alpha gene located in chromosome 17. Since in 95% of cases this gene is translocated with a promyelocytic leukemia gene in chromosome 15, the use of trans retinoic acid was surprisingly successful, even when not coupled with concomitant chemotherapy, curing 70% of the patients (*Camacho, 2003; Sell, 2004*). Furthermore, new research has brought up many other possibilities for therapies. As such, targeting two oncogenic events, one being IDH1/2 mutations and the other being the activation of FLT3 mutations (*Madan & Koeffler, 2021*), with different agents as therapy have also been approved for different types of acute myeloid leukemia (AML) since 2017, which serves as an example of how this types of therapy are still being developed and perfected nowadays.

Nonetheless, the use of this type of therapy for solid tumors has many obstacles. As noted in many research publications (*Cruz & Matushansky, 2012; Xu et al., 2014; Yan & Liu, 2016; de Thé, 2018*), there are many differences between solid and liquid tumors. First of all, the differentiation pathways that minimally vary in liquid tumors can be very different for solid tumors between types and patients, even if they are the same histological type. Secondly, solid tumors involve a high degree of cooperation between multiple oncogenic pathways, making them a much more complex ecosystem than the one present in leukemia (*Vogelstein et al., 2013*).

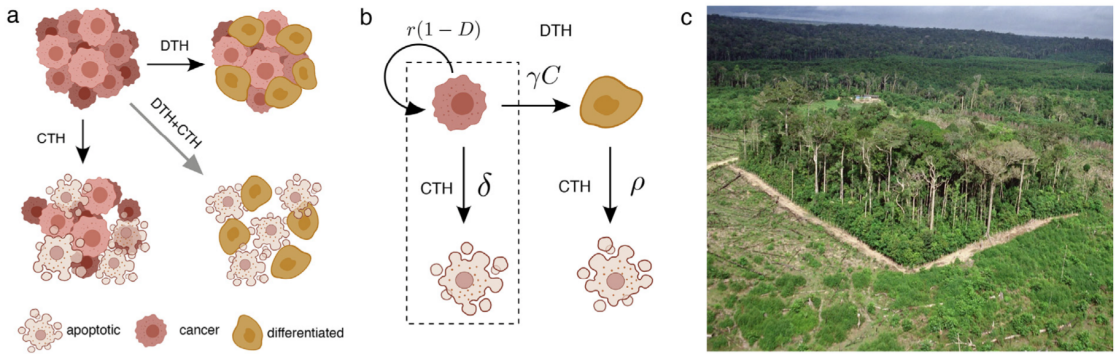

Figure 3: A metapopulation model of tumor differentiation therapy. In tackling alternative treatments to cancer progression, DTH exploits the potential of blocking tumor growth by activating differentiation pathways. A combination of cytotoxic therapy (CTH) and differentiation therapy (DTH) as shown in (a) can successfully kill the tumor when both separately cannot. In (b), both the complete model including the dynamics of the differentiated compartment and the minimal habitat-loss approach (dashed box) is shown. The DTH + CTH model is inspired in studies of habitat fragmentation (c), where habitat reduction, along with stochastic mortality, can trigger species extinction. *Solé & Aguadé Gorgorió, 2021*

Lastly, as they further elaborate in that study, the degree of heterogeneity present in solid tumors, such as lung tumors and melanomas, can be better appreciated by the fact that such tumors present about 10 times more non-synonymous mutations than leukemias do, further supporting the idea that liquid tumors present a much less complex landscape of aberrations in the genome, and a low degree of heterogeneity, making them a perfect fit for differentiation therapy. It should also be highlighted that many reagents have proven to be efficient tools in preclinical models, but that efficiency has not translated into significant results at the clinical level.

How does DTH work? Why only-differentiation treatments fail to deliver cure? Why it fails when dealing with solid tumors? The first two questions were tackled in (*Solé & Aguadé Gorgorió, 2021*) using an ecological conservation problem as seen in Figure (3) and its mathematical treatment as inspiration. They compared DTH with the effects of habitat loss and fragmentation (*Bascompte and Solé, 1996*) with a limited size and with a fraction D of destroyed area. They consider different layers of complexity in this spatially-implicit model to explain the leukemia results. The simplest one reads:

$$\frac{dC}{dt} = rC(1 - D - C) - \delta C \tag{1}$$

Here C stands for population (normalized), r and *delta* are colonization (growth) and extinction (death) rates, respectively. One key result from this model is that the nontrivial fixed point, namely

$$C_1^*(D, \delta) = 1 - D - \frac{\delta}{r} \tag{2}$$

is stable provided that habitat loss is lower than a certain D_c critical destruction

level. This result is somewhat unexpected, since we would conclude that the population will always be able to keep alive with the remaining $1 - D$ available space. That is not the case. These authors translated this concept to DTH combined with chemotherapy. If we map D to the fraction of differentiated cells, we can see that there is a qualitative agreement. What is more important, the basic clinical outcomes found from DTH treatment in liquid tumors seemed to match. The model thus predicts that, under some given parameter combinations, DTH should work. However, this is under the assumption of a homogeneous, well-mixed and thus non-spatial context. Can these extra factors be responsible for the failure of DTH in solid tumors? In (*Solé & Aguadé Gorgorió, 2021*) the authors also speculate that a tissue architecture where stem cells play a central role would more easily escape from DTH. Here we address these open questions using a spatially-extended discrete model grounded in Cellular Automata.

A Cellular Automata is a discrete regular grid of cells, where each one of its components can have a finite number of states. The simplest example would be a grid of cells where there are two possible states: on or off. Of course, this model can get as complex as the system it is supposed to mimic, which enables this to be a very powerful tool for the modelling and study of plenty of different matters, such as cancer growth itself. As a matter of fact, the study of tumor dynamics through CA has been done for a long time (*Williams & Bjerknes, 1972*), and more complex works related to this topic have also been developed, such as the study of interactions between tumor and immune system (*Mallet & Pillis, 2006*), and analyzing the possible outcomes that can result from treatment (*Monteagudo & Santos, 2015*). Though this complex simulations are far from perfectly mimicking cancer's behavior, since there are still many questions that need to be answered, this serves as a good approach towards a better understanding of not only the high degree of complexity that such ecosystems have, but also on how treatments can be oriented and designed towards a more efficient battle against cancer.

Taking into account the previously mentioned potential of cellular automatons, and the proven usefulness of reaction-diffusion models in the understanding of cancers such as glioblastoma (*Fort & Solé, 2013*), the purpose of this work is to analyze, through the creation of a hybrid model (different examples can be seen in Figure (4)), the dynamics of an avascular tumor, as well as studying the possible effects that differentiation therapy can have on such population. This approach takes into account the effect of space and ecology into the dynamics of differentiation therapy, as well as including the key role of CSCs in the model, which separates it from other models that have been developed to explore different therapeutic options, that usually do not consider the role of ecology and space. Intra-tumor heterogeneity will be minimally studied as well.

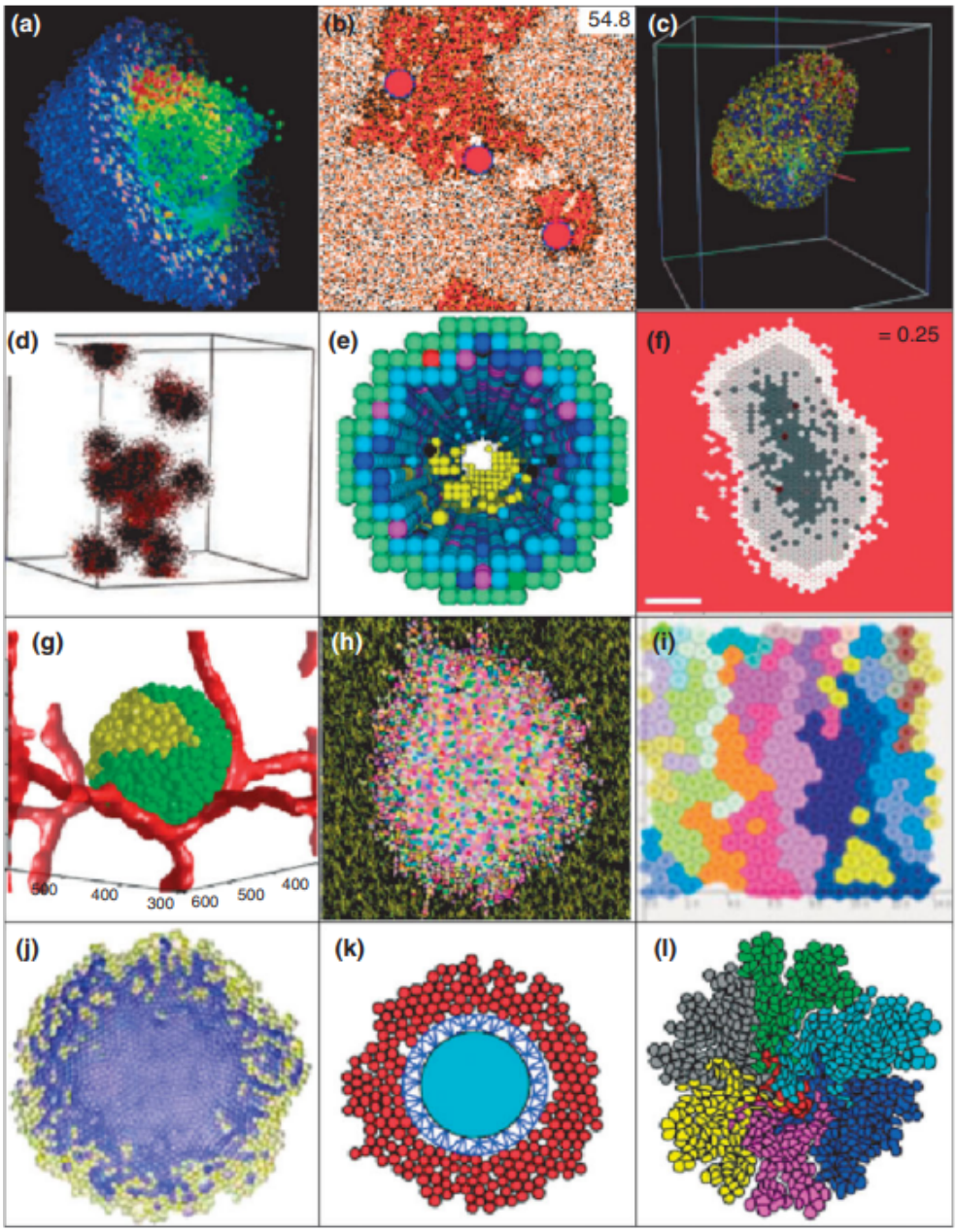

Figure 4: Snapshots from different simulations of hybrid models that use spatially-explicit modelling of tumor growth involving both discrete and continuous components. For further information regarding each image, see *Rejniak & Anderson, 2011* .

2 Methods

The overall development of the project consists in the creation of a cellular automaton capable of mimicking the growth of a tumor and its response to differentiation therapy. The nutrients available to the cells play a big role in the development of this population, since competition among cells is of great importance. The different parts conforming the overall CA are the following.

2.1 Tissue

The system intended to be mimicked will grow on a lattice Ω of cells. Such lattice will be of size LxL, with $L = 500$, and there are three possible types of cells that will be considered to exist in it: normal, cancer and tumor necrotic cells. For the sake of reducing the computational cost in our work, we will not consider the development of subpopulations inside the tumor in some cases (see the section on heterogeneity). Furthermore, each site $\mathbf{r} = (x, y) \in \Omega$ can only be occupied by one type of cell. Nevertheless, cancer cells, unlike the other types, will be capable of stacking inside the same space. Once there are no live cells left in one site, a single dead or tumor necrotic cell will be introduced. The different cell populations will be described as spatiotemporal variables: the density of normal cells will be indicated as σ_N, and they will be assigned the value $\sigma_N(\mathbf{r}, t) = 1$; cancer cells on the other hand have a population $\sigma_C(\mathbf{r}, t) = 1, 2, 3, \ldots$; tumor necrotic cells, symbolized as σ_D, will be given the value $\sigma_D(\mathbf{r}, t) = 0$.

The vessel from which the nutrients will be diffused is located at $y = 0$, which means that it will be placed along the horizontal axis. Furthermore, periodical boundary conditions are established along the vertical axis, while the horizontal axis will not have such boundaries, representing a delimited tissue in order to provide an environment closer to what we would see happen in a real tissue. Lastly, according to the clonal origin of cancer (*Iannaccone et al., 1987; Garcia et al., 2000*), one single cancer cell will be located at the center of the lattice, from which the subsequent tumoral population will develop.

2.2 Nutrients

A key component of our system is the presence of nutrients that will dictate the behavior of the population. As discussed by Scalendari (*Scalendari et al., 1999*), there are many nutrients that play a role in cancer growth. Elements such as iron and oxygen (*Weinberg, 1995; Delsanto et al., 2000*) are of the utmost relevance to favour cell growth. Hence, many different elements could be considered, and a large number of nutrient fields could be added to the model. Taking this into account, as considered by Ferreira *et al.* (*Ferreira et al., 2002*), the presence of two different types of nutrients will be considered in our model. On the one hand, there are essential nutrients such as oxygen and iron that promote cancer cell replication and survival, and such will be considered in N. On the other hand, other nutrients that participate mainly in cell motility and death will be encompassed in M. Both fields of nutrients will be considered to diffuse with the same coefficients of diffusion, and

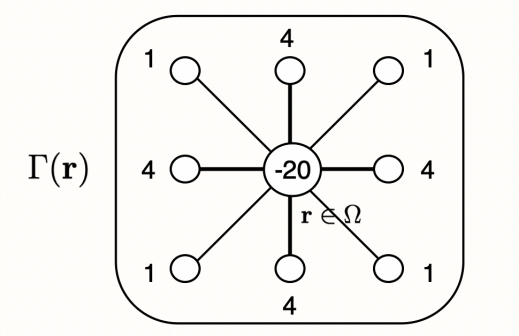

Figure 5: This scheme exemplifies the 9-point stencil employed for the calculation of the discrete Laplacian operator, with the relative contribution of each pair $(\mathbf{r}, \mathbf{r}')$ is indicated.

its uptake by normal cells will also be considered to be equal, but cancer cells will exhibit a higher uptake. The following equations emulate the diffusion fields of both nutrients:

$$\frac{\partial M}{\partial T} = D\nabla^2 M - \alpha^2 M(\sigma_N(\mathbf{r}, t) + \lambda_M \sigma_C(\mathbf{r}, t)) \tag{3}$$

$$\frac{\partial N}{\partial T} = D\nabla^2 N - \alpha^2 N(\sigma_N(\mathbf{r}, t) + \lambda_N \sigma_C(\mathbf{r}, t)) \tag{4}$$

Judging by the expression above, we can see that the consumption rates are proportional to the number of cells present in each site, since it is important to remember that different cancer cells can pile up in the same site. Furthermore, the coefficients λ_M and λ_N represent the difference in consumption rate between normal and cancer cells, being $\lambda_N > \lambda_M$, due to cancer cells' higher affinity towards essential nutrients that increase the replication capability in accordance with literature (*Weinberg, 1995; Ferreira et al., 2002*). It is also important to note that α is another parameter associated with the consumption of both essential and nonessential nutrients. Here D is the diffusion coefficient, considered to be the same for both types of nutrients. For this case, in order to decide which coefficient value to use, first we need to know what is the diffusion coefficient of molecules in the tissue. After some research (*Sniekers & van Donkelaar, 2005; McMurtrey, 2016*), the diffusion coefficient of small molecules and other larger proteins is assumed to be between 10^{-10} and $10^{-11} m^2/s$. Since the scale we are working with is between 0.1 and $0.01mm$, and taking into account the diffusion coefficients used in other models of reaction-diffusion (*Morgan & Kaper, 2004*), the diffusion coefficient established for both types of nutrients is $D = 0.05$. This also enables that the maximal concentration of the nutrient field is saturated to the unity. The discrete Laplacian operator used is thus one particular instance of the general model where a Moore neighbourhood $\Gamma(\mathbf{r})$ for each lattice site $\mathbf{r} \in \Omega$ is used:

$$\nabla^2 \phi(\mathbf{r}) \approx \frac{1}{h^2} \left[\sum_{r' \in \Gamma(\mathbf{r})} D(\mathbf{r}, \mathbf{r}') \phi(\mathbf{r}') - \left(\sum_{r' \in \Gamma(\mathbf{r})} D(\mathbf{r}, \mathbf{r}') \right) \phi(\mathbf{r}) \right] \tag{5}$$

where $\phi = M, N$ in our model description and h would be the minimal spatial scale (inter-site distance). In this work, a specific nine-point stencil[1] with given weights has been used (see Figure (5)).

The boundary conditions established are the following: $N(y = 0) = M(y = 0) = 1$, since as noted before, the vessel that will provide the tissue with nutrients is located along the horizontal axis. To account for the periodical boundaries along the vertical axis, $N(x = 0) = N(x = L + 1)$ and $M(x = 0) = M(x = L + 1)$ are the conditions established.

2.3 Cell behavior

Cancer cells present in the model will be able to carry out one of two different scenarios, each one of them having the same probability of taking place. The probability of each event happening is in accordance with the model proposed by Ferreira *et al.* (*Ferreira et al., 2000*).

The first event that can take place is replication. This process will have different outcomes depending on the situation of the chosen cell. If it is located inside the tumor (i.e. all of its neighbors are cancer cells), the daughter cell resulting will pile up on top of its parent in the same site. Otherwise, if the cell is located in the peripheral zone of the tumor (i.e. one or more of its neighbors are either normal or tumor necrotic cells), the daughter cell will randomly occupy one of the vacant sites. If this site was previously occupied by a normal cell, the cell will be assumed to have moved to another layer of the tissue, effectively being replaced by the cancerous cell. The probability of replication taking place will be a function dependent on the essential nutrients present in the site, as well as the number of cells present in it:

$$P_{div} = 1 - \exp\left(-\left[\frac{N}{\sigma_C \theta_{div}}\right]^2\right) \tag{6}$$

As can be extracted from the equation and seen in Figure (6)(a), the probability of division follows a Gaussian curve, due to the Gaussian term included. The probability is shown as a function of the number of cells in one site, considering the nutrient concentration to be maximum.

The second logical event that can take place is death. As explained before, tumor necrotic cells arise from previous cancer cells that die. That is controlled by the amount of nonessential nutrients present in that site. If there is not enough to assure cell survival, this process takes place. The probability controlling this event is the following.

[1] If the coefficient of diffusion is multiplied by the 9-point stencil, the grid obtained has -1 as center weight, 0.2 weight in the adjacent neighbors, and 0.05 in the diagonal elements. This resulting grid is the one considered to be most appropriate for reaction-diffusion models to avoid the appearance of artifacts. See https://www.karlsims.com/rd.html

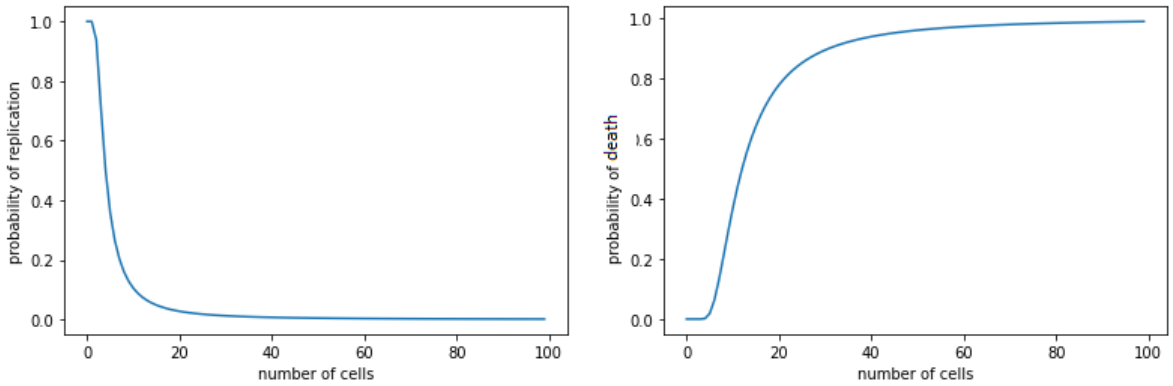

Figure 6: (a) Probability of division over the number of cells present in one site with $\theta_{div} = 0.3$.(b) Probability of death represented over the amount of cells present in one site with $\theta_{die} = 0.01$

$$P_{die} = \exp\left(-\left[\frac{M}{\sigma_C \theta_{die}}\right]^2\right) \tag{7}$$

Similar to the equation that defines the probability of replication, we have a Gaussian term that depends on the number of cells present in one site and the model parameter θ_{die}, as well as depending on the concentration of the non-essential nutrients. Figure (6)(b) is done with a low concentration of non-essential nutrients, 0.1, and plotted as a function of the number of cells present in one site. The event of cancer cells having the ability to migrate (*Björklund & Koivunen, 2005; Irimia & Tomer, 2009*) is something that takes place in real life and in latter stages of cancer development as metastasis, so it will not be considered in this work.

From the different probabilities, there are some conclusions that can be extracted. First and foremost, replication depends on the amount of essential nutrients available on that site and also on the number of cells present in it. As it has been explained, cells will only pile up when there are no empty spaces around it (i.e. the cell is located inside the tumor). Hence, division and subsequent cancer growth will be larger near the border of the tumor due to both large amount of nutrients and lack of competition for it. As pointed out in some experiments (*Brú et al., 1998; Brú et al., 2003*), this type of behavior is the one taking place in real-life: a constraint of cell proliferation to the tumor border, and growth within the centre of the tumor being greatly limited, since overpopulation would mean an increase in the competition for nutrients, being oxygen one of the main elements that will rapidly lack. This event can be somewhat overruled by the capability of tumors to develop different mechanisms that enable its population to reduce its consumption of nutrients (*Warburg, 1956; Sukumar et al., 2015*). This ability of cancer cells to be able to meet the biological requirement for tumor growth and proliferation is also known as the "Warburg effect", named after the person that first discovered such phenomenon. Nonetheless, for the sake of simplicity for this work, this phenomenon will not be considered. The same happens with the process of angiogenesis, in which

tumors develop vessels to better irrigate the different layers of the tumor, so that there is enough access to nutrients and events like hypoxia or even anoxia inside the tumor, which would lead to cell death, do not take place (*Adair & Montani, 2010*). Furthermore, the θ parameters, which are different for each probability, are a simplification carried out that include many different mechanisms and metabolic processes that regulate cancer's behavior. Most of these underlying mechanisms are still unknown (*Bourzac, 2014*), meaning that this interpretation is for simplification purposes only. Lastly, the cells present in the tumor will be considered to be undifferentiated, since a big part of their identity is the loss of differentiation, resulting in an increased potency. As a result, once therapy is administered, cancer cells will be assumed to differentiate into a differentiated cell, assuming only one level of differentiation for the sake of simplicity.

2.4 Therapy design

For the design of the therapy, there are not many papers that propose mathematical models for representation of differentiation therapy (*Solé & Aguadé-Gorgorió, 2021*). As a result, the modelling of this therapy has to be developed with no other similar approach with which to compare. In a model of a stochastic cellular automata (SCAM) developed by Pourhasanzade and Sabzpoushan (*Pourhasanzade & Sabzpoushan, 2019*), the authors developed a model of chemotherapy, affecting both the probability of replication and the probability of death due to the treatment. This was used for the basis of the development of my own differentiation therapy model.

So, the model is based upon the fact that differentiation therapy will affect cancer cells through two different mechanisms, one being the transition from poorly differentiated to differentiated cells, where they lose their identity and ability to replicate and generate new cancer cells, and the other being the decrease in the probability of replication of cancer cells. The latter is contemplated due to the fact that in the transition from undifferentiated to differentiated, the potency of the cell decreases (*Samsonraj et al., 2015*), which would translate in a diminishing in its probability of successfully replicating, while the alteration of the tumor microenvironment (TME) also affects the overall growth of the tumor (*Klein & Glazier, 2011*). Hence, the first mechanism is introduced through the presence of a probability of differentiation of cancer cells caused by the DTH therapy. Such probability is calculated by means of the following equation:

$$P_{diff} = L \cdot \exp\left[-c\left(\frac{t - n_d\tau}{\tau}\right)\right] \tag{8}$$

There are different terms that play a role in this equation. First we have the coefficient of attenuation c, which accounts for the attenuation of the drug once it is in the tissue. The second term is time t, which is a pretty straightforward variable, representing the time in the simulation after tumor detection. The starting time of therapy, denominated t_s, will be established afterwards, and its value is set after detection (t_{det}). The parameter n_d indicates the number of cycles the

patient has undergone the therapy. This parameter is of key importance, since the number of cycles may depend on the patient and the progression of the tumor. Some clinical trials (*Norsworthy et al., 2016*) have pointed out that patients could undergo up to 6 cycles of therapy of this kind. The intrinsics of these cycles - a mix of continuous and intermittent administration of drugs- are omitted in this case for the sake of simplicity. Furthermore, τ represents the half-life of the agent employed as therapeutic drug. Lastly, the parameter L is calculated as follows:

$$L = \frac{k}{n_d \gamma_{res} + 1} \tag{9}$$

Here k represents the theorized differentiation rate from the therapy, while γ_{res} represents the resistance that cells will show towards the treatment. As studies have shown (*Chlapek et al., 2018*), resistance to retinoids such as retinoic acid is one of the most common problems that may prevent DTH from successfully acting upon tumors. As a result, this term is introduced to account for that kind of behavior. Furthermore, the overall behavior of the probability of success of the therapy is tied to the previously mentioned resistance that cancer cells may develop to this treatment. As a result, the probability of success of the therapy decays over time following an exponential trend, altered by the cycles of drug administration.

The second mechanism involves the decrease in the capability of proliferation of cancer cells. If we consider equation (6), the resulting probability of replication under treatment follows:

$$P'_{div} = P_{div} \cdot \frac{\gamma_{res}}{n_d} \tag{10}$$

In this way, both the resistance of cells and the number of cycles of the treatment have an impact on the overall probability of division of cancer cells. Since the heterogeneity in concentration of the drug is not considered in this work, DTH will be assumed to be present everywhere uniformly. Hence, the objective of this work is to analyze how the tuning of different parameters may affect cancer growth, providing a deeper insight into the challenges that arise from this therapy, as well as analyzing its consequences and some case studies.

2.5 Cancer stem cells

One of the most important elements of this work is the presence of cancer stem cells. CSCs are characterized by their resilience and plasticity, which allows them to survive in an environment where other cancer cells would rapidly be suppressed via molecular signalling and other phenomena (*Wang et al., 2013*). They have proven to be one of the main factors that prevent chemotherapy or radiation from successfully eliminating a tumor (*Ishii et al., 2008*). Studies have shown the presence of hierarchical differentiation in solid tumors, a structure that reminds us of the classic hematopoiesis (*Ghiaur et al., 2011*). As a result, CSCs are capable of inducing tumor growth from scratch (*Batlle & Clevers, 2017*), which explains why tumors that are dormant or thought to be eradicated by chemotherapy or radiotherapy among other treatments can grow again, even more aggressively due to the culmination of

Darwinian evolution (*Peitzsch et al., 2017*). Nonetheless, they are not considered to have a higher replication rate.

Another important characteristic of CSCs is the amount of them that conform a tumor. As studies suggest (*Yu et al., 2012; Bao et al., 2014; Rich, 2016; Arnold et al., 2020*), the proportion of CSCs amongst a tumoral population covers a wide range, going from below 0.1% in AML and liver cancer to above 80% in other types such as acute lymphoblastic leukemia (ALL). Hence, the proportion of CSCs present in the tumor has been set to be 0.1% of the total amount of cells present in the tumor, and they will be assumed to be resistant to DTH. This is of key importance for the development of this work. That is due to the fact that resistance in stem cells arises not only from their cellular plasticity (*Foo et al., 2009; Sharma et al., 2010; Meachem & Morrison, 2013*), but also thanks to the unique characteristics and the role of ecology in the independence present among niches of CSCs (*Adams & Scaden, 2006*). It is also important to note that agents capable of effectively differentiating CSCs have not been found yet (*Xu et al., 2014*). Furthermore, CSCs will be assumed to sustain two different types of reproduction: symmetrical and asymmetrical (*Knoblich, 2010*). The former would mean that from one cancer stem cell, two daughter stem cells originate. On the contrary, the latter means that one daughter would be stem cell while the other wouldn't. Their distribution within the tumor is discussed in the Results section.

2.6 Heterogeneous population

The last remarkable element that has been contemplated in this study has been the introduction of an heterogeneous population. In solid tumors, the feature of heterogeneity is one of the most important factors that threaten therapies effectiveness. The wide variety of genetic aberrations present in solid tumors (*Jögi et al., 2012*) provides them with the weapons necessary to develop drug resistance (*Dexter & Leith, 1986*), making this one of the biggest threats to therapy's success. Furthermore, when it comes to differentiation therapy, the presence of different mutations translates into different pathways being affected, thus limiting the effectiveness of differentiating agents that focus on one particular pathway. As a result, a notion of how the degree of heterogeneity of solid tumors affects the chances of DTH successfully acting upon the tumor will also be introduced.

To account for such heterogeneity, the capability of mutation of cancer cells will be introduced. Since many mutations can take place, we will only consider mutations that confer resistance to drugs, in this case being agents used in differentiation therapy like retinoids (*Chlapek et al., 2018*). Such mutation rate is high in cancer cells, between 10^{-3} and 10^{-6} (*Duesberg et al., 2000*). Hence, three different mutation rates will be studied in this work, denominated by μ: 10^{-3}, 5×10^{-4} and 10^{-5}. For each subpopulation generated, a random factor between 0 and 1, ϕ_{res}, will be set. This parameter will then be employed as seen in equation (11). As we can see, this factor will therefore provide a sub-population with resistance to the treatment. The

smaller the factor is, the higher the resistance of one population to the treatment will be, since the probability of these cells being differentiated by DTH will be lower. Since the factor can be given a maximum value of 1, all populations will either be resistant or have the same sensitivity to treatment than the first population. Hence, deleterious mutations that increase cells' sensitivity to treatment will not be taken into account.

$$P_{diff} = L \cdot \exp\left[-c\left(\frac{t - n_d\tau}{\tau}\right)\right] \cdot \phi_{res} \tag{11}$$

2.7 Algorithm design

The algorithm has been implemented in Python 3. The first step of the process is to calculate the steady state of the nutrient field. Such state is found when there is no significant variation of the concentration in each site as time advances. As a result, a threshold for this difference has been established below which the nutrient field is considered to be in its stationary state. That threshold is 10^{-6}, which means that while there is a variation of concentration in one site $\mathbf{r} \in \Omega$, the entire field will not be considered to be in the steady state and the algorithm will keep iterating it. Once this has been cleared, the next step is to go through each cancer cell of the lattice separately. This can be done sequentially going one by one in order, but that might generate artifacts. In this work the updating of each cancer cell is done through a Monte Carlo method[2], which means that if there are x cancer cells in the lattice at a given time step, then x cancer cells are selected at random with equal probability, and one cancer cell can be updated more than one time in that time step. Once they are selected, the updating of the cancer cell is the next step. In it, there are two events that can take place as mentioned earlier, and they have the same probability of happening. Since each one of them involves the increase or decrease of the number of cells in one site, that means that the nutrient consumption is altered in that neighborhood. As a result, and in order to reduce the time taken to compute the nutrient field, an updating of the steady state of the nutrient field is done in an 11×11 square centered around the site affected by the change. The threshold used for this update is the same one as before, and this procedure allows for a faster computation of the steady state at the beginning of each time step. Once x cancer cells have been updated in that iteration, the entire procedure is repeated once again in the next time step. It is also worthwhile noting that the stationary state in the absence of any cancer cell (i.e. the entire lattice of normal cells) is considered for $t = 0$, and the case of heterogeneous population has only been considered in the subsection dedicated to it, while the other simulations have been done assuming one single type of cancer population. Lastly, the simulation stops once a cell reaches $y = 5$, since if a cell reaches $y = 4$, the nutrient field in that position cannot be recalculated with the requirements that have been established.

[2]Since each time step we update x cancer cells, the time step is thus proportional to such number, the increase in one step being $\Delta t = \frac{1}{x}$, following the Gillespie algorithm.

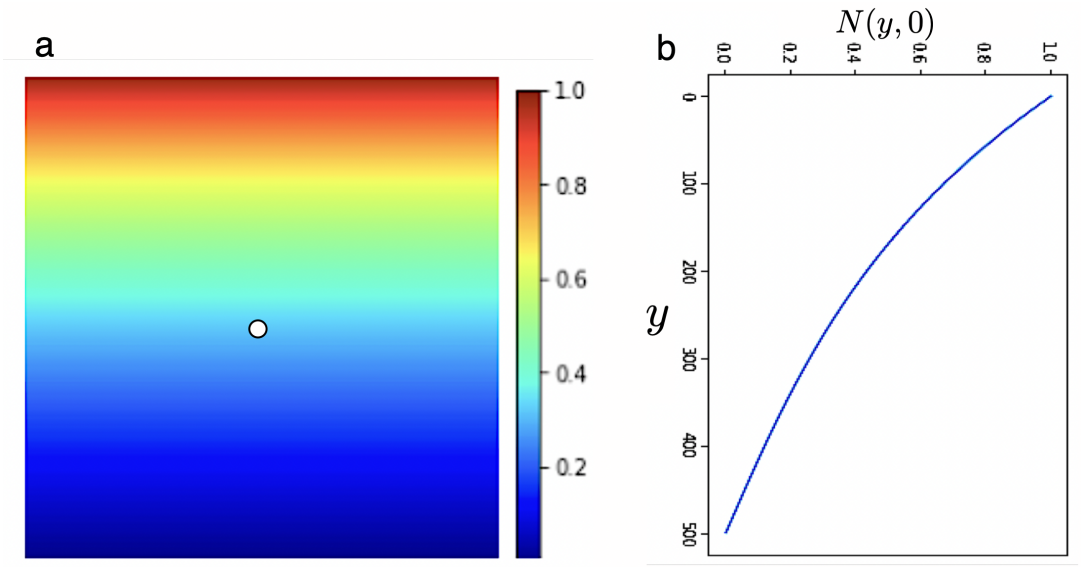

Figure 7: (a) Spatial distribution of the nutrient field at t=0 and $\alpha = 1/500$, in the absence of cancer cells (the initial location of the seed for the simulated tumor is indicated by an open circle). It defines an exponential decay in space over the vertical dimension. In (b) the corresponding linear profile $N(y, 0)$ is shown.

3 Results

3.1 CA Validation

Before introducing the results of the DTH therapy proposal, a proper validation of the CA needs to be done, comparing the results obtained in this work with the literature. As mentioned earlier during the explanation of the CA, the nutrient consumption by normal and cancer cells is a key factor to the development of the tumor. Taking into account that the parameter α accounts for the consumption rate of normal cells, and considering the multiplicative factors λ_M and λ_N, different types of tumors will be developed. One where the base consumption rate is low and the multiplicative factor will be gradually increased, and another one where the base consumption rate is high and the multiplicative factor is high as well, as proposed by *Ferreira et al.* As a sum up, the following values will be studied for each variable: α will be given a value of $1/500$ and $4/500$, λ_M will be given a value of 10 and λ_N will be given value of 25, 50, 100 and 200, each being studied for the lower value of α, while only the highest value of λ_N will be shown for the higher α, since no significant change was appreciated between different multiplicative factors for it.

Let us start with $\alpha = 1/500$. The nutrient field obtained for both essential and non-essential nutrients in the steady state at $t = 0$ will be the same for both cases since the consumption by normal cells is assumed to be the same. Figure (7) showcases the gradient generated across the tissue, and it can be noted that the closer to the vessel ($y = 0$), the higher the concentration of the nutrients is. Due to the fact that the nutrient consumption rate of cells is the lowest one studied, the gradient generated is not steep, but rather slowly decaying the further it gets from the ves-

sel. Once we have the nutrient field, the next step is to analyze the tumors generated.

As it can be seen in Figure (8), the increase in consumption by cancer cells has a significant effect in the overall behavior and spatial distribution of the tumor population. In the runs shown here, the tumor cell populations in the snapshots are: $n = 193.065$ cells for the tumor with the lowest multiplicative factor λ_N, and $n = 69.828$ for the largest. This can be better understood by visualizing the resulting essential nutrients field for both cases. As expected (see Figure (8e-f)) a clear correlation with tumor density is present. For lower λ_N, the overall concentration of nutrients is more uniform and the zones were consumption is high, the nutrient field is lower in the case of higher consumption rate. This simple fact provides the basis for understanding one of the most important hallmarks of cancer, which is their metabolism reprogramming to reduce their need for nutrients, acquired both via mutations and the tumor microenvironment itself (*Cantor & Sabatini, 2012; Yang et al., 2017*). Without this advantage, nutrients become scarce in highly populated regions, meaning that their ability to reproduce in the nucleus of the tumor is drastically reduced. This also helps understand why most proliferating cells can be found in the tumor border (*Brú et al, 2003*).

Of course for this to be of relevance, this type of compact tumor needs to be compared to real-life tumors. This is actually on of the reasons we choose this particular implementation to explore the DTH problem. Two examples are shown in Figures (8g-h) that illustrate the two limit cases considered in Figure (8a,d). One is a more compact (but asymmetric) shape, as illustrated by the basal cell carcinoma, and the second has a more papillary-like branched structure, as shown in the trichoblastoma. If we look back at Figure (8a-d), the higher the multiplicative factor λ_N is, the more papillary-like form that the tumor develops. It is also remarkable how this simple models are able to recreate one fundamental characteristic of not only tumors, but cell populations as a whole: the development of colonies that are fractal[3] (*Cross et al., 1995; Losa, 1995; Brú et al., 2003*).

Figure (9) shows the population dynamics that tumor growth follows. One would expect that the dynamics of the population will follow exponential growth (*Murphy et al., 2016*). Nonetheless, in the early stages of development of the tumor, its growth does not follow the exponential-like form, but rather a slower growth shape. That is due to the fact that there is a competition for nutrients that limits the ability of growth of the tumor[4]. Furthermore, since the concentration of nutrients in the early stages is far from maximal, since it is located far from the source of nutrients, the chances of replication for cells are limited and as a result its growth will be slow, and as they get closer to the source (i.e. at time t=700), nutrients are

[3]Although not explored here, the presence of these scale-invariant shapes are relevant to understand spatial tumor complexity. These fractal shapes typically stem from amplification-inhibition processes driven by diffusion under limited resources.

[4]Additionally, despite the fact that the number of cells can grow locally (as a result of the failure of contact inhibition) spatial dynamics is always limited by the local nature of interactions. In general, a trade-off between local advantages (nutrient accessibility) and the proximity to the tumor boundary lead to slower-than exponential regimes.

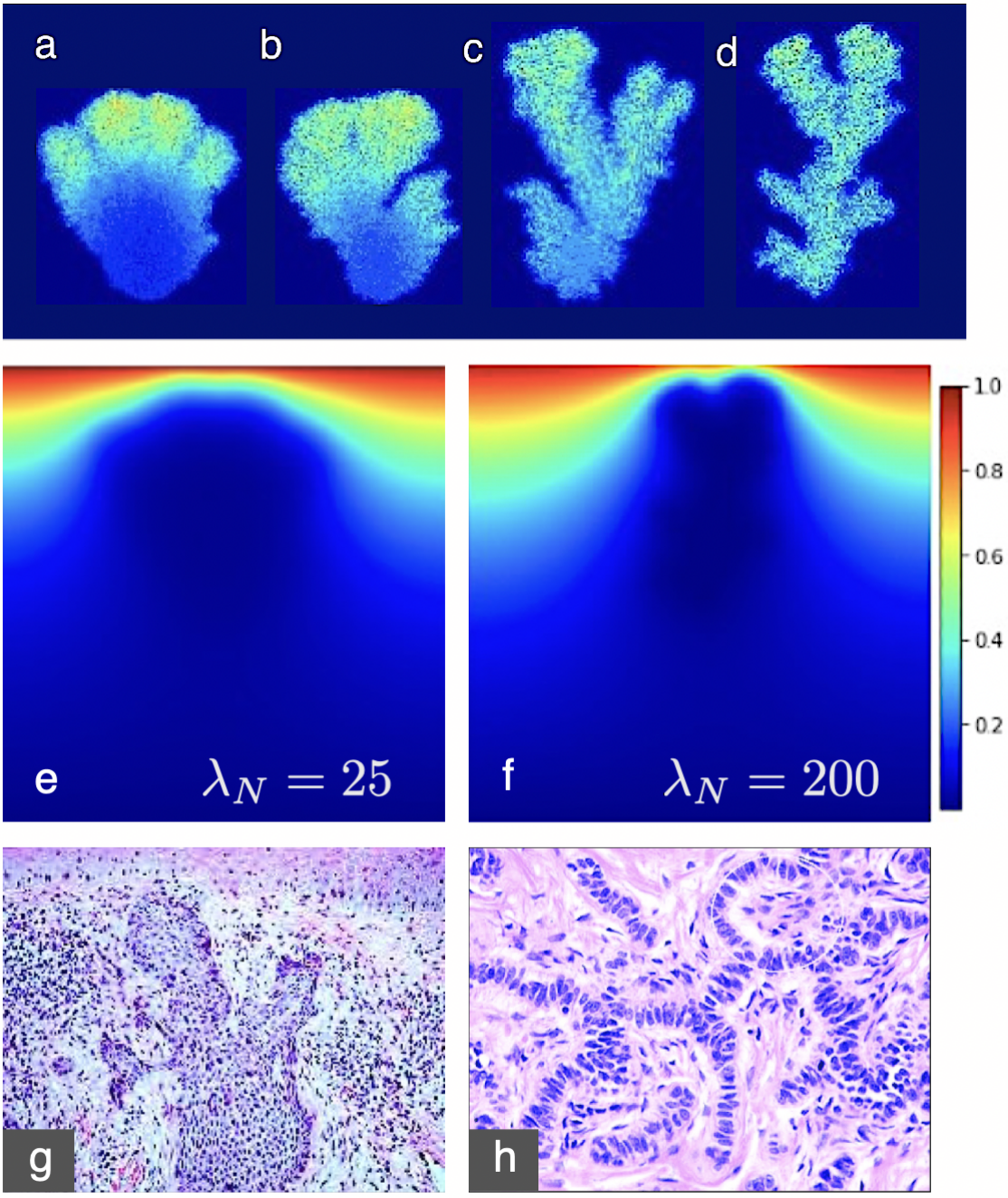

Figure 8: Tumor growth with different multiplicative nutrient consumption factors **a** λ_N=25, image **b** to λ_N=50. Image **c** is obtained with λ_N=100 and image **d** with λ_N=200. The two extreme cases (**e**,**f**) of the resulting nutrient field are shown in the lower figures. For comparison, we show two microscope images corresponding to (**g**) a basal cell carcinoma (from Yale university archives see https://medcell.org/histology/skin_lab/basal_cell_carcinoma.php) displaying a more compact (but non-symmetric, closer to (**a**)) spread and (**h**) a branched structure characteristic of so called trichoblastomas (closer to (**d**); from *de Vico et al., 2011*).

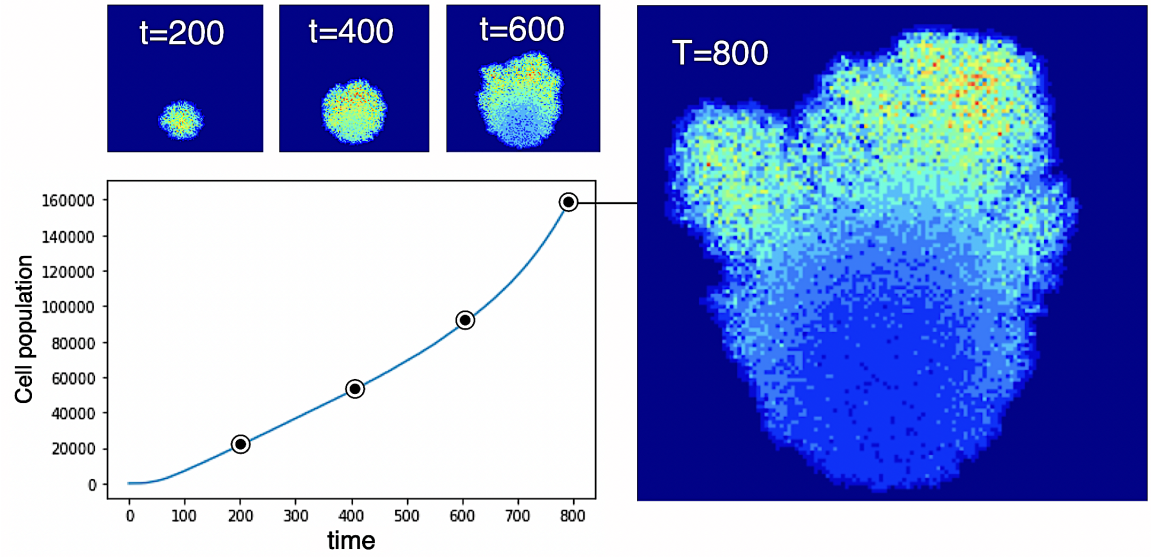

Figure 9: Population dynamics in space and time for a tumor using λ_N=25. Four snapshots are taken at different times of the simulation and their location within the growth curve is indicated.

abundant, which enables cells to replicate faster than in early stages, achieving that exponential growth previously mentioned in latter stages. Nonetheless, this rate of growth cannot be infinitely maintained, and in the previous study mentioned they predicted through a Gompertzian model that, eventually, the tumor will reach a stable point, growing to a given maximum size.

An important factor discussed earlier is the capability of cancer cells to not only change their metabolism, but to also promote angiogenesis (*Carmeliet & Jain, 2000*), which is the formation of new vessels within the tumor in this case. This process can be triggered by a number of mechanisms, some of which are metabolic and mechanical stress as well as mutations (*Carmeliet, 1999; Kerbel, 2000*). Hence, this hallmark is not taken into account nor modelled in this study, due to the high degree of complexity behind it since there is a balance of activation and inhibition molecules (*Nishida et al., 2006*) that regulate and exacerbate this phenomenon. It may be possible that the addition of it to the model drastically changes the population dynamics, and it should be a target moving forward, with some novel studies (*Owen et al., 2009*) that should serve as a starting point.

The impact of high consumption rates on tumor spatial complexity is clear from (10) where a fractal pattern is observable in the rugged shape of the tumor boundary. As some studies note (*Brú et al., 2003*), fractal growth was proven to be a key factor in different tumors of different size and cell type, while they also noted that this growth is not compatible with Gompertzian growth, but rather more linear growth regimes[5]. This may also explain why, in the early stages of the tumor, the

[5]Mathematically, linear growth can result from the dominance of boundary-related effects, where tumor growth is limited to the boundaries. If we approximate the external boundary of the tumor

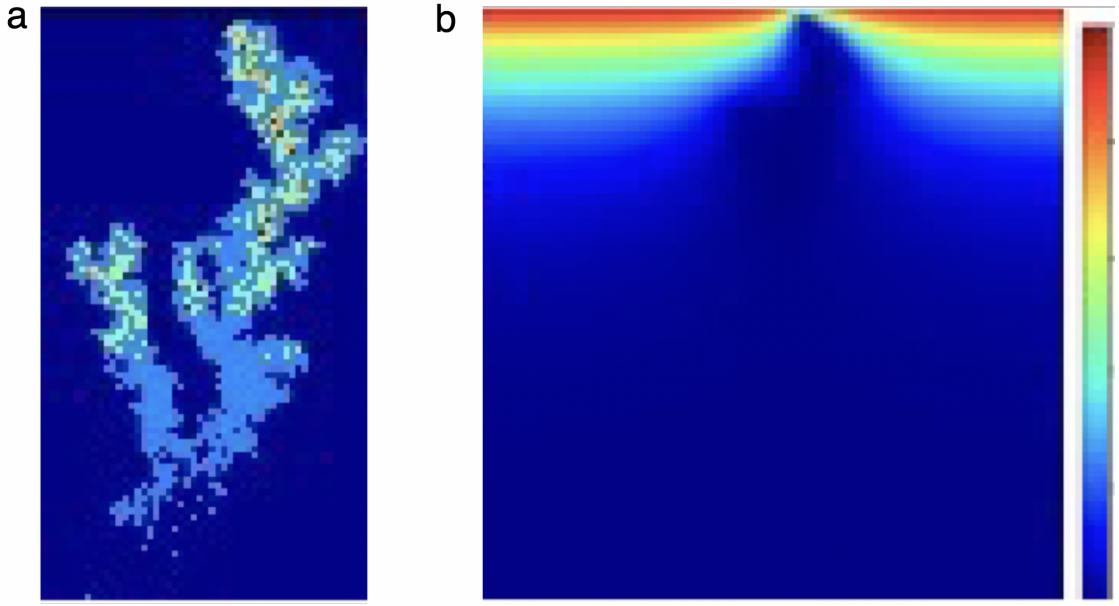

Figure 10: Fractal-like patterning of tumours under high consumption rates (both the tumor (a) and the corresponding gradient (b) are shown), with $\alpha = 4/500$ and $\lambda_N = 200$

growth seen in Figure (9) looks closer to a linear growth regime than Gompertzian growth. Furthermore, high nutrient consumption is also associated with limited growth of tumors (*Chen et al., 2009*), and this is supported by the fact that the total population of the tumor is considerably less large than the other cases.

3.2 Treatment

As explained in the Methods section, one important factor to take into account is the number of cycles of therapy given. As it was also mentioned, the maximum patients were given was 6 cycles. Hence, that will be the value assumed. It is also important to note that t_{det} (considered time of detection) will be set at 300, while t_s (start time of therapy after detection) will be set at 100, and CSCs will be assumed to create distributed niches. This is of key importance and the effect that this has on the overall growth of the tumor will be studied afterwards. Nonetheless, many studies have shown that CSCs niches are either developed thanks to the tumor microenvironment that provides the necessary factors, or they occupy pre-existing, tissue-specific stem cell niches (*LaBarge, 2010*). As a result, and as noted in the

as a circle, the biomass of it will scale as $B(t) \sim c\pi r^2$ where c stand for the biomass per unit area. From this relation, we can derive the functional relation between radius and $r = c\sqrt{B}$ with $c = 1/\sqrt{c\pi}$. If growth happens only in the peripheral part of the tumor, it will be proportional to $L = 2\pi r$ and thus growth $\approx 2\pi c\sqrt{B}$. We can write an equation for the dynamics of tumor biomass as:

$$\frac{dB}{dt} = \mu\sqrt{B} - \delta B \tag{12}$$

where we use $\mu = 2\pi c$. It can be shown that this model leads to a linear growth dynamics.

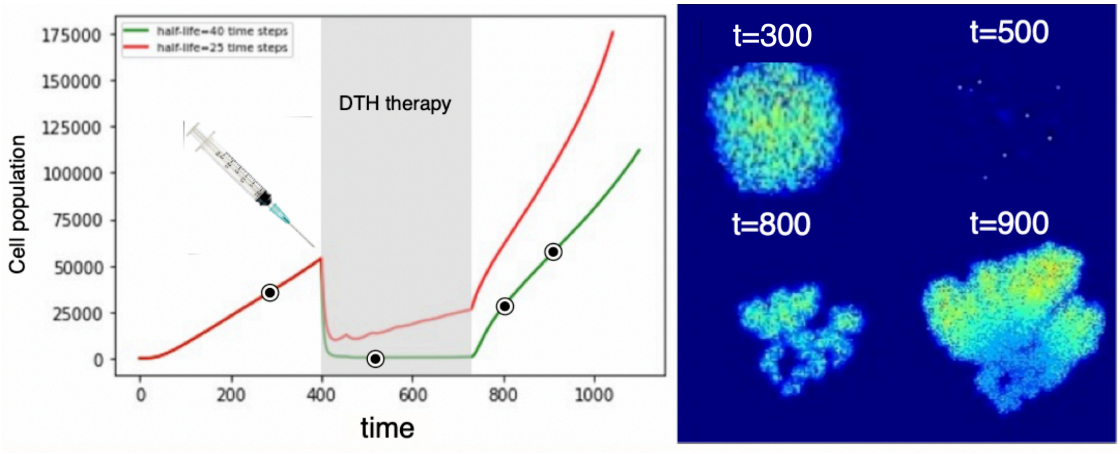

Figure 11: A comparison of the effect that the drug's half-life (τ) has on the dynamics of the tumor population, with half-life of 25 (red) and 40 (green) time steps respectively compared. The parameters employed are $\gamma_{res} = 0.55$, $t_{int} = 55$, $k = 0.8$ and $c = 0.5$, and the grey zone represents the time interval in which therapy is active. The tumor shown on the right side are snapshots taken from the growth of the tumor under treatment with a half-life of the drug of 40 time steps, and the different stages of progression can be seen.

previous study, the quantity and location of CSCs niches in a tumor varies within a wide range, making it difficult to accurately create a picture of it, which is the reason why they will be randomly placed. Apart from that, three different parameters will be studied: τ which is the half-life of the drug, t_{int} which is the interval of time between cycles, and γ_{res}, which represents the resistance from cancer cells to the treatment. Furthermore, the effect of the distribution of CSCs niches has also been studied with a given set of parameters, studying both from the point of view of a local CSC niche, and niches being distributed across the tumor. It is also worth noting that all simulations for treatment start with a minimally developed compact tumor, with no more than 37.000 cells, and heterogeneity is not contemplated in this part.

The first results presented are the population dynamics depending on the half-life of the drug employed. As we can see in Figure (11) the results are somewhat expected, since as we can see in equation (8) an increase in τ translates into an increase in the probability of success (differentiation of cancer cells) of the therapy. The tumor grows after therapy due to the survival of CSCs.

Now let's study some not so elemental behaviors that can be obtained when tuning other parameters. In this case, the parameter in question is t_{int}, which represents the interval of time between cycles of administration of the drug. Taking $\tau = 40$, and the previous values given, the results are quite interesting. When analyzing Figure (12) there is an interesting behavior that can be seen in the effect that different intervals of cycles have on the overall efficiency of the treatment. We can see that the longer the interval is between cycles, the more affected the overall growth of the tumor is. Furthermore, results seem to show that it is in the best

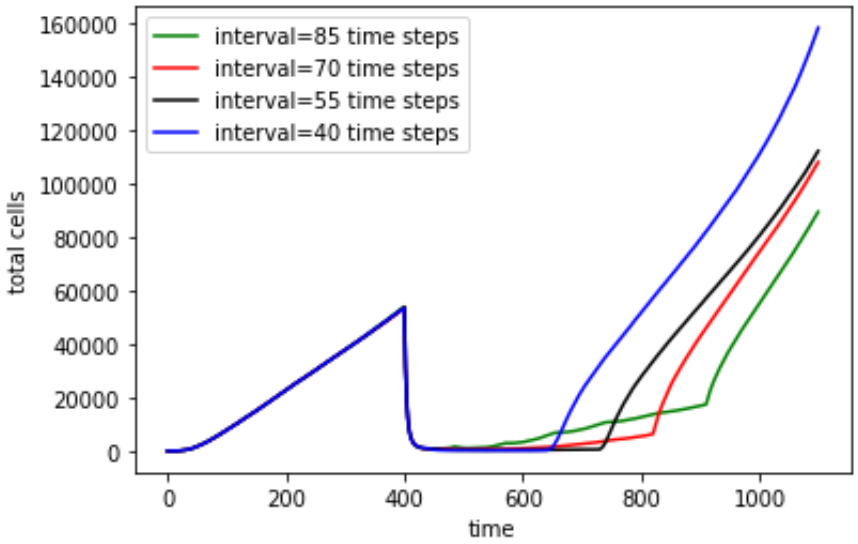

Figure 12: The effect of different DTH time spans is shown. Since we only give 6 cycles of therapy, different intervals of administration result in more or less time the tumor is under treatment. When dynamics show great increase in growth, that means therapy has already been stopped. The parameters employed are $\gamma_{res} = 0.55$, $\tau = 40$, $k = 0.8$ and $c = 0.5$. The total time in which treatment is active varies as a result of different time intervals between cycles.

interest of the patient to have longer intervals of time between cycles, which seems to be in accordance to what clinical studies have shown for chemotherapy (*André et al., 2020*), which is the fact that longer duration of therapy has an overall increase in 5-year survival rate of patients with stage III colon cancer for instance. Nonetheless, a major factor they noted is the increase of toxicity tied to a long period of therapy, as well as a usual increase in cost. However, this toxicity registered in chemotherapy does not have to necessarily mean that DTH will have that same effect. Studies regarding the use of differentiation therapy in oral cancer (*Meyskens et al., 1991*) showed that some agents employed for DTH were associated to no side-effects. Carotenoids showed to have no toxicity effects in the long term, while all-trans retinoic acid (ATRA) proved to generate toxicity. Further studies (*Tarantillis et al., 1994*) have also noted that the toxicity associated to ATRA is something worth monitoring for its use in high doses, while the low or non-existent degree of toxicity in carotenoids may allow it to be used in high doses without major concerns.

Another important factor that needs to be taken into account is the overall resistance of the tumor population to differentiation therapy. In equation (9) there was the parameter γ_{res}, that symbolizes the overall resistance of cells to DTH. As it can be seen in Figure (13), a logical result is obtained, where a higher resistance by cells translates into a less successful effect by therapy, since more cells are able to overcome it.

The last important difference that will be analyzed, and which was previously mentioned, is the effect that the location of CSCs has on the overall behavior of the

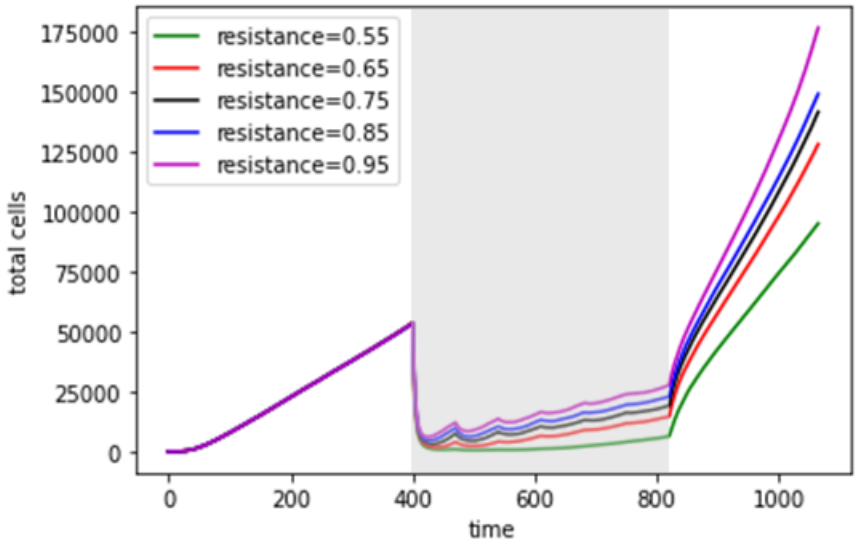

Figure 13: The cell resistance of cancer cells under DTH is compared. The parameters employed are $\tau = 40$, $t_{int} = 70$, $k = 0.8$ and $c = 0.5$, and the grey zone represents the time interval during which treatment is active. A clear correlation can be seen between the average resistance of the cells and the overall progression of the tumor.

tumor. The comparison will be made between cases with the same set of parameters, but one will have the CSCs randomly distributed in different niches among the tumor, while the other one will have its CSCs located in a single square niche of $6x6$, with 2 located in one site so that we have 37 CSCs, because at the time of detection the tumor has around 37.000 cells. Niches are specific regions of the tumor where cells inside it create a microenvironment that provide factors that enable CSCs to have the capacity of constant self-renewal, as well as inducing angiogenesis among other things (*Plaks et al., 2015*).

Figure (14) serves as an example of how space and density of population may affect CSC's capability of proliferating. It is well known that CSCs usually inhabit highly-differentiated niches (*Voog & Jones, 2010*), which creates an ideal microenvironment that gives them access to plenty of different factors that have a significant impact in proliferative potential. As it can be seen throughout the different simulations carried out, the tumor ends up growing again, and that is due to the fact that, as established before, CSCs have been assumed to be resistant to DTH. Hence, eventhough during treatment most of the tumor is eradicated, the survival of CSCs allows for the re-population of the different tumors, which is considered the main factor in tumor growth and relapse (*Enderling et al., 2013*). This resilience and ability to initiate and develop tumors comes from the unique environment provided by the niches that has been previously mentioned.

Nonetheless, a component many works fail to account for is the amount of nutrients available. As noted in many studies (*Hanahan & Weinberg, 2000; Sottoriva*

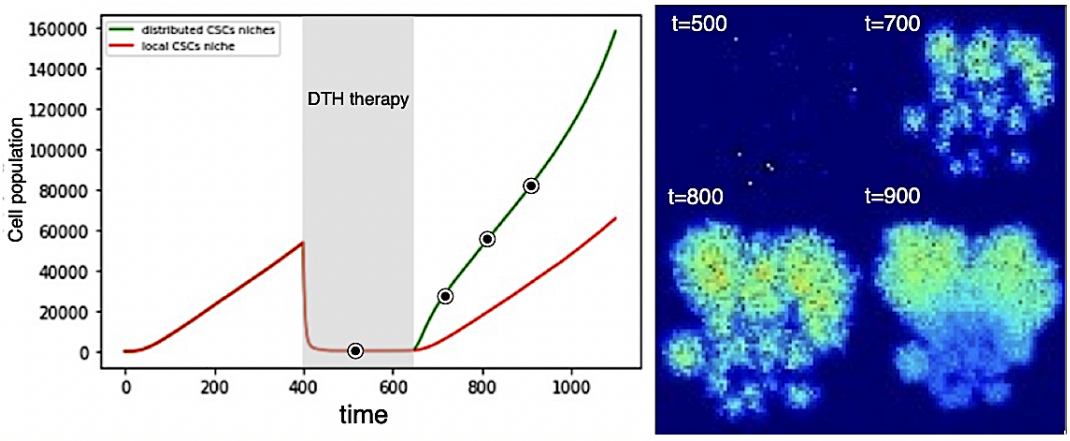

Figure 14: The effects that the location of the CSCs. In the different stages of development of the tumor after the treatment when assuming distributed niches of CSCs (green color on the left), we can see that different colonies are created from CSCs, since they have more access to nutrients and less competition for it in early development, and they can rapidly grow. The set of parameters employed are the following: $\gamma_{res} = 0.55$, $t_{int} = 40$, $k = 0.8$, $c = 0.5$ and $\tau = 40$.

et al., 2010; Finicle et al., 2018), the nutrient concentration plays a major role in tumor growth and invasion amongst others. As a result, if we consider that therapy at one point differentiates almost all cells except for CSCs, if CSCs are distributed among the tumor they will have less competition for nutrients, which will result in a greater tumor growth than in the case where CSCs are grouped in a single niche.

Furthermore, one can see that if distribution of niches of CSCs is assumed, each one of them can give place to small colonies at first, separated between themselves, meaning that there is a great amount of nutrients they have access to, allowing for a greater tumor growth in its early stages, being more aggressive. The role of ecology in the niche habitat, as well as its distribution and independence has been noted in some studies (*Adams & Scaden, 2006*) as a key contributor to the threat niches pose, while tumor plasticity has also been noted as a key contributor to the elusiveness of tumors (*Plaks et al., 2015*). Furthermore, CSCs extinction might not prevent the tumor from eventually regenerating them from non-CSCs while under treatment (*Chaffer & Weinberg, 2015*).

However, there is another component that is of great importance in tumor development: the effect that crowding the CSC niche has on the overall replication capability of CSCs. This factor may affect CSCs capability of proliferation by slowing it down, but this effect has been difficult to capture (*Vainstein et al., 2012*). Nonetheless, due to the limitation by nutrient availability, crowding effects may take a toll in the overall capability of CSCs to replicate in this work. As it can be seen in Figure (14), a more densely populated niche has a lower proliferation rate than less densely populated and more distributed niches, successfully capturing the behavior previously mentioned. In fact, many studies (*Betteridge et al., 2006; Dingli & Michor, 2006; Enderling et al., 2009; Hillen et al., 2013*) have discussed the accel-

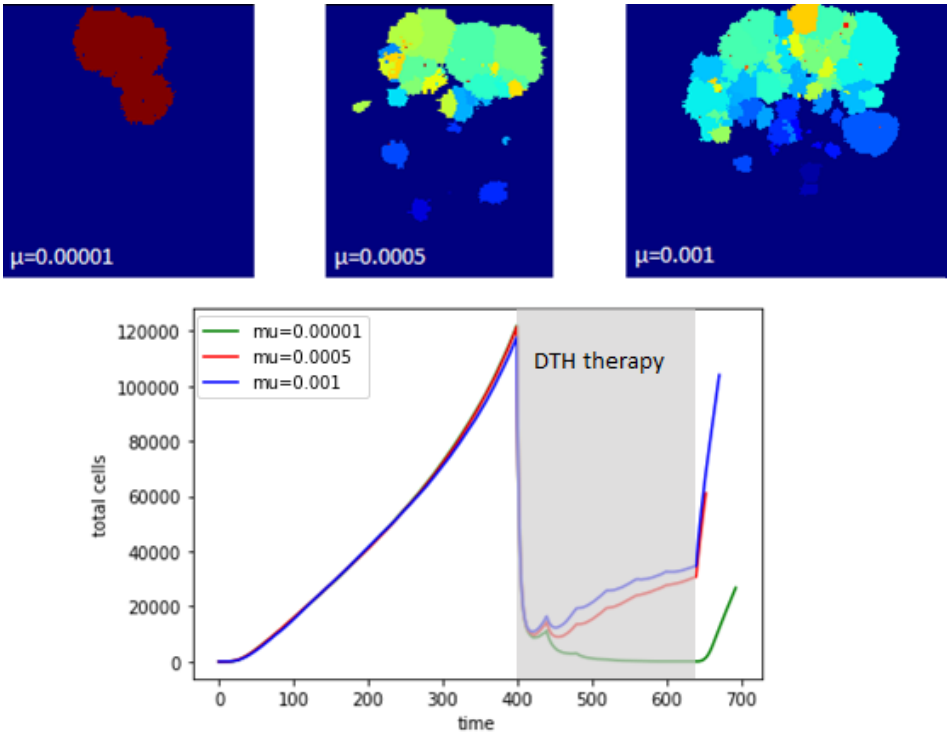

Figure 15: An example of the impact of population heterogeneity on the overall dynamics of the tumor when treated with DTH. The parameters are: $\gamma_{res} = 0.55$, $t_{int} = 40$, $k = 0.8$, $c = 0.5$ and $\tau = 40$.

erated tumor growth that can be seen when competition for nutrients decreases, be it due to cell death as a result of crowding (*Picco et al., 2016*) or cytotoxic therapies or cell differentiation through differentiation therapy, both of which take place in this study. Hence, this paradox can be seen with different treatment approaches, where low density of population after treatment leads to an even more aggressive progression of the tumor, something that has been captured in this model.

3.3 Heterogeneity

In this section, the effect of DTH on a heterogeneous population will be studied. As was mentioned in the Methods section, three different values for μ have been studied, and since there is no limit to the different subpopulations that can be generated, the population dynamics under treatment could significantly change from one scenario to another. In Figure (15) we display the dynamics of the population under treatment with different mutation rates. On the one hand, the population with the lowest mutation rate ($\mu = 10^{-5}$) exhibits a behavior similar to the one we already described (see Figure (14)), where the tumor is suppressed when placed under therapy, but it grows again afterwards. Nonetheless, in this case, its growth is limited due to the fact that such population reached the limit of the lattice Ω in the simulation. This is interesting because only one new population was originated in this simulation, but it was created near the border of the tumor, due to the fact

that cancer cells near the border of the tumor are the ones proliferating to a large extent, while the ones located in the centre of it do not proliferate (*Brú et al., 2003*). Nonetheless, their resistance to DTH was low, which is the reason for their almost complete eradication under treatment.

If we analyze the case with a higher mutation rate ($\mu = 5 \times 10^{-4}$), we can already see a substantial change compared to the former case. Many different populations arise as we can see in Figure (15), which at its turn increases the probability that a new subpopulation has greater resistance to DTH. That is the main reason why, when the tumor is placed under treatment, the drug-sensitive population is eradicated while the drug-resistant population survives and grows. That is also helped by the fact that as sensitive cells die, competition for nutrients diminishes, which in turn allows resistant subpopulations to grow faster. This behavior is the same one seen in the case with the largest mutation rate ($\mu = 10^{-3}$), where the chance of resistant subpopulations developing is greater. Nonetheless, the growth of both populations under treatment is very similar, with only the better-fit strains being able to survive.

4 Discussion

In the present work a preliminary computational model has been developed, capable of characterizing the behavior that derives from the interaction between a tumor and differentiation therapy, as well as studying different factors that have an influence in the overall result. The approach taken has provided some very interesting results that correlate to the experimental observations, such as the effect that the duration of therapy has on the overall behavior of the tumor, while also giving some new insights into the importance of ecology and competition in tumor growth.

The motivation behind the development of this framework lies on the effectiveness that differentiation therapy demonstrated when treating APL. The use of chemotherapy and radiotherapy for the treatment of solid tumors have long been established as the best possibilities for many types of tumors. Nonetheless, a cytotoxic therapy of this kind can have some undesirable side-effects (*Howell & Shalet, 1998*), and sometimes therapies are not effective enough to completely obliterate the tumor, with its success largely varying amongst tumor type and stage (*Ashdown et al., 2015*). These matters are the main reason why exploring new options and studying the possible outcomes are of great importance towards taking new approaches to fight back cancer.

In this context, differentiation therapy is one promising option. Its effectiveness has been proven in the treatment of liquid tumors, like AML and APL. However, its effectiveness is tied to the low intratumoral heterogeneity present in liquid tumors (*Xu et al., 2014*), with APL being characterized as a simple karyotype disease that can thus be eliminated with the reversal of that specific pathway. Nonetheless, solid tumors are viewed as heterogeneous masses that evolve and develop new mutations as time passes, with a hierarchical distribution of cells and CSCs at the top of it,

which are capable of triggering heterogeneity and repopulating the tumor when it has been eradicated. As a result, a higher degree of complexity is encountered when trying to design an effective treatment for them. Hence, the classic mono-target approach taken to treat AML for example cannot be taken, but rather a more diverse approach, either combining differentiating agents, or the combination of differentiation therapy with chemo or radiotherapy. This approach was not studied in this work, but rather some key points on the effect of DTH in solid tumors in order to better understand its weaknesses moving forward, which include the previously discussed crowding effect, which can be a potential therapeutic target since this pathway of key importance in tumor development and relapse as has been explained..

4.1 Habitat fragmentation: the ecological picture

The construction of niches that end up being densely populated has proven to be a limiting factor for CSCs proliferation, not completely obliterating it but rather slowing down their capability to do so, being this an important therapeutic target (*Dingli & Michor, 2006*). As shown in the results section dedicated to that matter, a niche with a high density of population is suboptimal for the development of tumors, since there is high competition for nutrients and for space, while more distributed niches can achieve greater success due to the resulting ecological picture described. Regarding the microenvironment generated in those niches, CSCs have access to a wide variety of factors that enable them to maintain that endless ability of self-renewal, while also enabling the tumor to reach some very important hallmarks, such as tumor angiogenesis for instance. As a result, this polyvalence is of key importance in tumor relapse (*Najafi et al., 2019; Marzagalli et al., 2021*), since CSCs are able to endure treatment and repopulate the tumor afterwards. Studies also show that placing cells under survival-threatening conditions contributes to the development and expansion of such CSC phenotype (*Pattabiraman & Weinberg, 2014*), while also accelerating the development of angiogenesis, as was pointed out earlier. This last step is of key importance to the survival of the tumor, since it can be seen from the model that excessive consumption of nutrients will eventually be unsustainable for proper growth of the tumor. As a result, the focus should be shifted towards finding therapeutic options that can efficiently target angiogenesis, which would be an efficient blow to cancer's chances of success. Although this topic has been recently put in the focus of attention (*Bid et al., 2013; de la Torre et al., 2020*), there is a long road ahead before some substantial improvement and clinical trials can be performed. Nonetheless, models like the one developed in this work can be of great help towards that end.

4.2 Aggressiveness of therapy and its consequences

The aggressiveness of therapy is an important matter to take into account. The time interval between cycles and the dose given are considered to be the most determining factors for it, and we only studied the former. A low time interval between

cycles, as analyzed in Figure (12), reduced the tumor population to mostly the CSCs, with the rest of it being differentiated, and maintains it that way for some time. In this interval, selective pressure is further placed upon cancer cells (*Cahill et al., 1999; Komarova & Wodarz, 2005; Merlo et al., 2006; Ovens & Naugler, 2012*). As it was noted before, the intratumoral population of solid tumors has a higher rate of mutation than liquid tumors and, as a result, a more heterogeneous mass develops. Furthermore, the presence of different niches of CSCs also plays a role in this heterogeneity, since their capability of asymmetrical replication leads to the development of different populations among the tumor. As it was also noted before, the almost complete elimination of all non-CSCs cancer cells has an important impact in the ecology of the system: there is almost no competition for nutrients, and stem cells can easily proliferate at its highest rate while there is no overcrowding in its microenvironment. Nonetheless, as the time interval between therapy cycles increases, more non-CSCs are able to endure treatment and stay alive, therefore increasing the amount of competence for nutrients that CSCs have to face in order to survive. It is also important to note that, as explained in the Results section dedicated to Heterogeneity, we can see how the tumor slowly grows under treatment conditions, since many resistant subpopulations slowly compete for resources between themselves. Hence, a larger interval between cycles would also theoretically add competition with drug-sensitive cells to the equation. This scenario can be of key importance for the development of successful therapies to fight solid tumors. To better understand how important this is, let's introduce adaptive therapy. This approach looks to exploit the competitive interaction that arises between drug-resistant and drug-sensitive cancer cells (*Gatenby et al., 2009; West et al., 2020*), since the presence of drug-sensitive ones will suppress the proliferation of the less-fit drug-resistant cells. Hence, this approach looks at enforcing a stable tumor where drug-sensitive cells predominate, achieved through a treatment that is constantly modulated. Considering the spectrum of possibilities that this opens up, and taking into account that a bigger separation between DTH cycles allows for the survival of drug-sensitive cells, a combination of adaptive therapy and differentiation therapy could be a plausible and potentially efficient approach to tackle some of the most concerning aspects of solid tumors, like CSCs and intra-tumoral heterogeneity, and that should be studied moving forward.

4.3 Future work

Since this was a simplified model used to study the dynamics of DTH on an avascular tumor, there are many different things that can be expanded. Firstly, only two types of nutrients have been considered in the system, an oversimplification of the different nutrients that play a role in cell growth (*Yuan et al., 2013*). A more complex study would take into account different nutrients, and each would have a specific effect on cells. Secondly, the behavior for cells only involved the capability of division and death. Nonetheless, other options such as migration that enables metastasis could also be considered for the development of further studies. It is also important to highlight that in this work, a simple version of differentiated, non-

differentiated cancer cells is assumed. Nonetheless, future studies could potentially include different levels of cell differentiation. Furthermore, the probabilities of each event taking place have been designed according to the elements present in the hybrid model. Nonetheless, the θ parameters encompass many different factors not considered in this work, like cell-cell interaction, as well as considering the effect of the tumor microenvironment in the overall behavior of the population. As was pointed out before, hallmarks such as the development of angiogenesis should be considered to more accurately capture real-life behavior of the tumor, and to better understand how to approach that problem.

Regarding the approach taken to consider therapy, there is one main factor that affects the behavior of the therapeutic drug administered, and that is the pharmacokinetics (PK) (*Thurber et al., 2013*) associated to that drug. PK provides relevant insight into how that agent behaves once it is inside the human body, ranging from its absorption and distribution to its elimination from the organism. Since that is not accounted for in this work, further studies should also assess the effect of it in the overall effectiveness of the therapy. Lastly, CSCs have been assumed to be completely resistant to DTH, but the entire microenvironment that is generated in the CSC niche has been overlooked, and is still largely unknown, so further investigation in this topic should be done in order to more accurately model and better understand how these niches develop the resilience and inaccessibility that makes them hard to tackle.

5 Conclusion

The present work explores the feasibility to apply differentiation therapy (DTH) to tackle solid tumors. Although DTH has been shown to offer a powerful cure for some liquid cancers, its limitations within the context of solid masses requires both an explanation and insights that can help future treatments to succeed. In this context, the model presented here is a first step towards a basic science of DTH as well as a hypotheses generator. The spatial model dynamics has been fitted (within the current limitations) to available data and sets the basis for further study of DTH and how can it be used against solid tumors. As it occurs within the study of ecosystems, we have shown that the presence of spatial degrees of freedom places serious constraints to tumor dynamics that are not present under well-mixed (mean field) approximations. Future work should exploit the Achilles heels of such spatial constraints to enhance the potential of differentiation-based strategies.

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