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by

Mario Basevi[2], Pier Mario Biava[1*], Lucio Biggiero[2], Antonio Borgonovo[3], Emanuele Borgonovo[3*], Francesco Burigana[4]

[1]Foundation for Research into the Biological Therapies on Cancer, IRCCS Multimedica, Milano

[2]Knownetlab Research Group and University of L'Aquila, L'Aquila, Italy

[3]Eleusi Research Center and Department of Decision Sciences, Bocconi University, Milan, Italy

[4]AMEC- (Medicine and Complexity Association), Trieste

*Corresponding Author:

Name: Pier Mario Biava

Add/ Affiliation: [1]Foundation for Research into the Biological Therapies on Cancer, IRCCS Multimedica, Milano

Fax:

Tel:

Email 1: biava@tiscali.it

ABSTRACT

The recent tumor research has lead scientists to recognize the central role played by cancer stem cells in sustaining malignancy and chemoresistence. A model of cancer presented by [44] describes the mechanisms that give rise to the different kinds of cancer cells like-stem cells and the role of these cells in cancer diseases. The model implies a shift in the conceptualization of the disease from reductionism to complexity theory. By exploiting the link between the agent-based simulation technique and the theory of complexity, the medical view is here translated into a corresponding computational model. Two main categories of agents characterize the model: 1) cancer stem cells and 2) differentiation factors. Cancer cells agents are then distinguished based on the differentiation stage associated with their malignancy. Differentiation factors interact with cancer cells and cen, with varying degrees of fitness, induce differentiation or cause apoptosis. The model inputs are then fitted to experimental data and numerical simulations carried out. By performing virtual experiments on the model's choice variables a decision-maker (physician) can obtains insights on the progression of the disease and on the effects of a choice of administration frequency and or dose. The model also paves the way to future research, whose perspectives are discussed.

Keywords: Cancer Research; xxx; xxx; Agent-Based Modelling; Simulation; Computational Methods in Biology.

1. Introduction

Current research has evidenced the role of cancer stem cells in determining the evolution of the disease [47-87]. There has also been an effort of conceptualizing the biological behavior into a new theoretical model of the tumor. The model foresees a sequence of differentiation stages of the stem-cells, from the most malignant to cured. The steps of the differentiation are determined by the different factors that constitute the epigenetic code that regulates the gene-expression.

The purpose of this work is to introduce a computational model that reflects this recent paradigm of tumor research. The first question we face is the identification of the simulation method capable of reflecting this new conceptualization. We show that the agent-based simulation technique allows one with the necessary flexibility to capture the different aspects of the problem.

We build a model corresponding to the research performed in [44]. As we are to see, main elements of the model are, besides agents (stem cells and differentiation factors), the matching probabilities (or fitness), doses and frequency of administration and other characteristic biological parameters. We fit numerical values of the model by matching in-vitro experimental data in [39]. The model is then utilized to perform virtual simulations. Insights that support medical decision-making are discussed.

The remainder of the paper is organized as follows. Section 2 presents a literature review of the medical research relevant to this work illustrating recent advances. Section 3 presents the model of cancer, from a medical viewpoint. Section 4 illustrates a review of agent-based simulation in biology and in cancer research. Section 5 presents the simulation model created in this work and discusses simulation results and medical insights. Section 6 offers conclusions and perspectives of future research.

2. Cancer Cells as undifferentiated cells and the influence of stem cell differentiation stage factors on their growth.

The evidence obtained by studying the interactions between tumor cells and embryonic tissues suggests that tumor development is reduced or suppressed by the embryonic microenvironment [1, 2]. It has been discovered the following: a) the administration of carcinogenic substances during organogenesis causes embryonic malformations, without leading to tumor formation in the offspring; b) conversely, when organogenesis is complete, the administration of carcinogenic substances increases the frequency of tumors in offspring [3, 4, 5]. These data have lead researchers to conceive cancer as a deviation of the normal cell development, that can be controlled by factors present in the embryonic microenvironment during cell differentiation. Many years ago it has been demonstrated that the injection of embryonic carcinoma cells into a blastocyst is able to regulate cancer cells with loss of their malignant behavior [6]. This experiment has next been confirmed by other authors [7,8]. Further researches have evidenced that this effect is position-dependent: carcinoma cells placed in the perivitelline space have not lost their malignant behavior, while carcinoma cells injected into the blastocele lost their malignancy [9,10]. In [11, 12] further research on this aspect has not confirmed these data and the regression effect has been ascribed to diffusive factors and to the complex networks of substances of the embryonic microenvironment. For some time it has been thought that this effect is peculiar to embryonic tumors. However, [13, 14, 15, 16] have demonstrated that other types of tumors, including leukemia, melanoma, liver, breast cancer can revert into a normal phenotype and/or differentiate into normal tissue when implanted in the embryo. Most recently it has been demonstrated that the implantation of a melanoma in a zebrafish embryo, as opposed to an adult zebrafish [17, 18, 19], does not give rise to tumors. The injection of the melanoma in the extraembryonal membrane of the zebrafish has lead to the development of cells of the nervous system, showing that tumor cells have the ability to differentiate into normal tissue of the embryo [20]. However, these experiment have evidenced the following fate of tumor

cells: either the malignant cells are induced to die and to arrest growth [21], or they are committed to differentiating pathways [22]. Previously, [23] showed that kidney carcinoma nuclei obtained from frog can be reprogrammed, when introduced into an oocyte; a similar effect has been obtained in mice in which nuclei from a medulloblastoma implanted in embryonic cells were able to redirect early development. These results demonstrated that epigenetic regulation of tumor nucleus by nuclear transfer into an embryonic microenvironment is able to revert the malignant phenotype: the transplanted tumor nuclei gave origin to post-implantation embryos undergoing tissue differentiation [24]. These results are in accordance with those of other authors [25], who injected leukemia cells into the placenta of then-days-old mouse embryos and obtained mature animals with circulating leukocytes carrying leukemia cell markers. These data suggest that both the maternal (placenta) and embryonic elements constitute a morphogenetic field able to induce leukemia cells to differentiate. Similar results have been reached in other experiments [26,27], and it was possible to conclude that embryo-mother cross-talk is very important in determining the arrest of tumor growth, because both maternal (decidua) and embryonic tissues contain substances with anti-cancer properties [28]. Therefore, these promising results have opened new perspectives not only in cancer biology comprehension, but also in leading to a different therapeutic strategy. In fact, recently, further experiments have clarified that embryonic cells share fundamental features with tumor cells [29,30], such as: proliferation and expression of embryonic proteins (AFP,or ABC transporters), common molecular signals and pathways (i.e. beta-catenin/TCF/WNT Notch, BMP and Hedgehog signals) [31,32,33], anaerobic metabolisms [34] etc. In addition, results in [35, 36] suggest that the epitelium-mesenchimal transition observed in cancer tissue may also be viewed as a “reactivation” of an embryonic program: it has now well confirmed that tumor cells express genetic programs of immature or embryonic cells, as highlighted by genome-wide analysis of gene expression profiles of cancer tissues and embryonic stem cells [37, 38]. In the light of these facts, we also recall the experiments in [39] that demonstrated that factors taken from zebrafish embryos during precise differentiation stages caused a significant slowdown in the growth of various tumor types. In

addition, a significant decrease in Lewis lung carcinoma injected in C57BL/6 mice treated with differentiation factors has been observed by [28]. Studies to determine which regulation pathways are involved in this mechanism of tumor growth inhibition have demonstrated that key-role cell cycle regulator molecules, such as p53 [40] and pRb [41], are modified by transcriptional or post-translational processes. Research on apoptosis and differentiation has revealed that treatment with stem cell differentiation factors induces caspase 3 activation, mainly by increasing the release of E2F-1, leading to c-Myc overexpression and the activation of a p73 apoptotic-dependent pathway. Moreover, a concurrent significant normalization effect on the ratio of e-cadherin and beta catenin, with an increase in e-cadherin levels, was observed [42]. Finally, a product prepared for human therapy containing stem cell differentiation stage factors demonstrated 19.8% regression, 16% stable disease and a significant difference in survival between the group of patients who responded to treatment versus the group with progression of disease in an open randomized clinical trial on 179 consecutive patients with intermediate-advanced hepatocellular carcinoma [43]. These works confirm the importance in regulating tumor growth of factors present at specific stages of cell differentiation and allowed [44] to conceive a model of cancer based on the idea that malignancy of tumors is linked to cancer cell like-stem cells. Before entering into the analytical description of the model let us analyze the medical implications of this new tumor concept. In the last years, an huge number of researches demonstrated that the malignancy of tumors is linked to the presence of cancer stem cells [45], so that a complete overview is out of the scope of this work. We however, recall the results obtained in various types of solid tumors, such as glioblastoma [46,47,48] breast cancer [49, 50, 51, 52, 53,54], lung cancer [55,56,57,58], prostate cancer [59,60,61], ovarian cancer [62,63,64,65,66], liver cancer [67,68,69,70,71,72], gastric cancer [73,74,75,76,77], colon cancer [78,79,80], pancreas cancer [81,82,83], squamous carcinoma of the head and neck [84,85,86,87]. In these studies, it has been evidenced that malignancy and chemoresistance are due to the presence of cancer cells like-stem cells. On the other hands it is well known from a long time that the presence of cancer stem cells is characteristic of many hematological malignant disorders.

In the next section, we enter into the details of the medical model of tumor mentioned above.

3. The model of cancer.

The model of cancer that conceptualizes the results of the researches and experiments described in Section 2, has been introduced in [44]. It addresses the mechanisms that give rise to the different cancer cells like-stem cells. In this model, cancer cells are defined as cancer cells like-stem cells, blocked in a step of the multiplication process, comprised between two stages of cell differentiation. This happens because a cancer cell is seen as an undifferentiated cell in which the genetic and epigenetic alterations do not allow the cell to complete the whole program of development and in which the programs of multiplication and differentiation are uncoupled: in other words, a cell is "in a loop" repeating always the same instructions. Considering that the stages required to complete the whole process of cell differentiation are five (1° stage: from totipotent to pluripotent stem cells; 2° stage: from pluripotent to multipotent stem cells; 3° stage: from multipotent to oligopotent stem cells; 4° stage: from oligopotent to differentiating cells; 5° stage: from differentiating cells to the complete differentiated cells), it is possible to sustain that, regarding the malignancy, the most malignant tumors are represented by cells with gene configuration present in early stages of cell differentiation. It should be remembered that the current classification of tumor is redundant, because it does not consider that the most malignant types of tumor are constituted by cells holding the same gene configuration, as it is described in [44]. We note that the model provides a theoretical framework that encompasses the results of many experiments, which have been described in Section 2. These experiments, have shown that cell differentiation is a key-process in explaining the behavior of both normal and cancer stem cells. Cell differentiation mechanisms are based on a multigenic regulation, so that a more differentiated cell differs from a less differentiated one because of the expression of a great number of genes [44]. Therefore by providing cancer cells with the factors that can bring them to differentiation, tumor cells can revert to a normal phenotype. During the differentiation process the whole repertoire of regulators is present (transcriptional, post-

transcriptional, translational and post-translational factors, etc.) and able to differentiate specific kinds of stem cells at specific development stages. We have seen that these factors can be used for regulating gene expression and proteins configuration. Furthermore, if one wants to regulate the expression of many different genes, one has to use a network of differentiation factors, network that has to be complete enough. As a result, the focus should be on the microenvironment and networks of the biological structures, rather than on the single subjects of punctual mechanisms. Indeed, this leads us to a new scientific paradigm that is shifting our views from reductionism to complexity.

4. Review of Agent Based Simulation in Cancer Research

The use of agent-based simulation models (ABM) in biology and medicine is rapidly diffusing, so marking a growing acknowledgment of its relevance as a fundamental tool of research and experimentation. A clear sign of this trend is the proliferation of papers that explicitly address this approach or use it to test some theory or empirical procedure. Moreover, the publications are "percolating" from highly specialized journals, like the Journal of Mathematical Biology or the Journal of Complexity or Computational Biological Medicine or Theoretical Biology and Medical Modelling, to more diffused journals like BioSystems, the Journal of Theoretical Biology, Immunological Reviews, Vaccine, etc.

As we stated in the introduction, at the basis of the new paradigm of tumor research is the shift from reductionism to complexity. Complexity theory conceives the behaviour of a system as the result (or consequence) of the behaviour of its parts (or agents). This intuition applies to all those scientific problems for which it is not possible to come to a rational formulation of the system's objective function (Lagrangian, Hamiltonian, Utility function), that is, in all those cases, in which is not possible to derive the motion equations for the systems parts or components from a bottom-up perspective. Conversely, complexity theory rests on the concept that system behaviour can be still described, but utilizing a bottom-up approach, namely by obtaining system behaviour as a result of the laws or rules followed by the agents in the system. This explains is widespread utilization in

sociology, political science, demography and environmental sciences (e.g., [88], [89]; [90]). These premises are exactly the ones at the basis of an agent based model. It is widely recognized in the community that for social and biological systems the statement of the system's motion equation is currently unfeasible, at least based on the present state of knowledge.

Furthermore, ABM is particularly appropriate for accomplishing the task of creating a modular but comprehensive model of the tumor for the following reasons. (1) ABM focuses upon the behavior of agensts as part of a complex systems. It accounts for the rules according which they act and interact. (2) It has been demonstrated by many authors that ABM is capable of handling agent heterogeneity. (3) ABM makes it possible to integrate diverse disciplinary perspectives. A particular portion of the system architecture can be described by a separate set of mathematical equations that describe the behavior of the "agent" there represented. (4) ABM allows to structure the software architecture across the different levels of analysis and to explicitly reveal in the model one's understanding of the rules with which agents interact.

ABMs are the peak of a methodological revolution that is crossing social, natural, and artificial sciences [91, 92]. Its roots date back to the automata studies in the fifties (von Neumann), continued with the conceptual and practical experiments of von Foerster in his Biological Computer Laboratory at Urbana-Champaign. The strongest impulse was given by the approach of artificial life (Cowen, Holland) at the Santa Fe Institute in New Mexico (US), because the growing computer power allowed simulating complex phenomena through cellular automata [93]. Among those contributions Kauffman's approach of random Boolean networks [94] has had one of the major impacts on both the communities of biologists and economists. Finally, the development of advanced object language software allowed the rapid and currently fast growing generation of multi-agent models, among which do place the agent- (or even called, individual-)based simulation models [95, 96].

What they are concretely and how do they distinguish from mathematical simulation models, which also developed during last decades (and actually even preceded ABM)? They are “computational tools”, which can “reproduce”, that is generating a complex general problem or a specific phenomenon. The fundamental epistemological idea underlying this methodological approach is that the ability to reproduce the problem or the phenomenon implies that of explaining it, and consequently of predicting it future development. So, the model is made of agents (“objects”) that represent the – not necessarily concrete – elements constituting the problem, whose “behaviour” is described through equations, algorithms, and other logical and mathematical connections. Indeed, regardless of whether the problem at hand pertains to the social or biological science, a behaviour consists, at the very end, in the specific ways in which a certain agent interacts with the other agents, or with the environment (food, energy, data, etc.) or even with himself (his memory).

Once the problem is reproduced through the model, we get an extraordinary tool for research and experimentation: a virtual (or in silico) laboratory. Hence, we can explore the many combinations deriving from the interactions of agents by means of virtual experiments. Compared to the real experiments there is a number of advantages, and, of course, some disadvantage. The first advantage consists in the speed at which it is possible to run the experiment, and to analyze it. This can lead to time savings of three or four order of magnitudes. The second advantage is that the results have the strength of theorems, because an agent-based model can be seen as an axiomatic system whose products are just theorems. Of course, the empirical validity of such results-theorems depends on the validity of the model and on the calibration and validation of its parameters. The third advantage is that building an ABM forces the researcher to an extreme clarity of his ideas about the problem under modelling. The fourth advantage consists in the relatively easy reproducibility and extendibility of results, especially whether the source code of the computer program were made publicly available in some sort of open access style. In fact, much easier than with in vivo or in vitro experiments, the in virtuo experiments can be replicated and eventually developed. Consequently, the knowledge gained through a certain model could be easily capitalized

and compared with others. Finally, there is an invaluable advantage which happens apparently in medicine: “wrong” experiments do not damage anybody. Just “virtual patients” would die or suffer for “virtual therapies”. Especially into the field of oncology, such a property of this methodology should be considered mostly valuable.

The major disadvantages are that building, calibrating, validating, planning experiments, and interpreting results is all but easy. These tasks require time, expertise, and a strong collaboration between the modellers and the researchers: basically, teamwork should be employed. Moreover, such difficulties grow with the complexity of the model, which, in turn, replicates the complexity of the problem under investigation. Indeed, as it is occurring for all forms of bioinformatics, biological and medicine laboratories more and more require inter-disciplinary skills and heterogeneous theoretical and methodological perspectives.

It has been evidenced that agent-based models present advantages with respect to other tools, like system dynamics, network analysis, and Boolean networks, which form the currently crowded field known under the large umbrella of bioinformatics or computational biology. Very briefly, pure mathematical simulations use traditionally systems of differential equations, each one representing a certain class (or type) of agent. The appeal of this approach resides stems from the availability of algebraic solutions. However, this availability is at stake in real-world problems, because these problems are most often too complex to be treated and solved via differential equations.

Network analysis is very easy to implement, and its use, attractiveness and applicability are fast growing in biology, and especially in proteomics and genomics thanks to the availability of good software and databases. Some limitations are imposed by its static nature, that prevents one from obtaining insights into the causes of a given structure, and from making predictions about its future behaviour. Moreover, it cannot take into account complex or multiple neither relationships nor node attributes. Thus, though it is a valid method of research in biology and medicine, which is receiving further impulse from recent contributions even into the field of cancer research [97, 98,

99, 100], it appears severely limited when compared with ABMs. In respect to AMB, however, network analysis main appeal resides in the ease of use.

Though it can be used also in cancer research [101, 102], the approach based on (random or probabilistic) Boolean networks overcomes the problem of being static. In fact, the dynamics of a biological system is evidenced by its path and its attractor. The price one pays, however, is a strong simplification of the system elements and their interactions. These latter should be direct and limited to only one type, and the system components should be limited to two-states behaviour, like activated or inactivated. In respect of network analysis the gain in making the process dynamic is traded against the complexity of the system description. In this respect, ABMs have, at least in principle, no limitation in dealing with the complexity of the components (agents) and their interactions. Moreover, network size is dynamic. Several (perhaps most?) of empirical phenomena in social and natural sciences are made of networks whose size varies with their evolution. ABM limitations are generated by in practical aspects related to the complexity of modelling, calibrating, testing, analyzing and interpreting results. These issues, and thus, the choice of the appropriate degree of complexity, strictly depend on the model objectives.

On this base it is possible to suggest one among the many ways to categorize ABMs: the level of abstraction, which distinguishes between abstract, middle-range, and specific (facsimile) models [103]. The former aims at discovering general laws or phenomena holding beyond contingent instantiations, which nevertheless could eventually modify the outcomes. At the opposite extreme, models can be built for studying very specific cases for which they search a very high level of realism able to make reliable predictions. However, it should be remembered that even in very specific models all relevant aspects can be hardly taken into account. As in the logic of any model, the problem should be simplified, and it is necessary to start from the clearest situations. But despite of empirical (in vivo or even in vitro) experiments, in the in virtuo experiments we cannot exploit unspecified contextual conditions. This aspect has of course also a positive side, because we are

sure that no unspecified variable has affected our results. In other words, we perfectly control our experiments. In the middle lie models seeking explanations useful for entire classes of phenomena, and thus striking a balance between generality and detail. In fact, the first category of models should follow the so-called KISS (keep-it-simple-stupid) principle [103], while the third the so-called KIDS (keep-it-descriptive-stupid) principle [104]. The model presented in this paper belongs to the first category, since it simulates a general type of interaction processes between a very stylized form of cancer proliferation and drug.

Though it is definitely impossible in few rows to review the already vast literature of ABM in biology and cancer research, in what follows a brief sketch is provided. Besides a rapidly growing number of models applied to various problems, like the effect of intercellular signalling via the epidermal growth factor receptor on cell proliferation [105], the representation of acute inflammation [106], the protein-protein interaction network [107], the intracellular chemical reactions [108], etc., there are two main streams of research into the applications of ABM in biology and medicine: cancer and immunology. This latter started quite early and nowadays several models of the whole immune system have been developed [109, 110, 111, 112, 113,114,115,116,117,118,119,120]. Here, we actually find representatives of the three categories of models just discussed. The models of the whole immune system are the most abstract due to its complexity and to the relatively short time of study. The models for the analysis of the competitive interactions between the humoral and the cellular branch [121], the concerted action of B cell selection mechanism [122], and the vaccine efficiency [123] can be categorized as middle-range models. The simulation of affinity maturation and hypermutation in the humoral response [124] and the dynamics of human immunodeficiency virus HIV [125] can be listed among the category of facsimile models.

Into the stream of cancer studies most contributions are, with few exceptions [126], in between the middle-range and the facsimile models. Chen et al. [121] study cancer cell motility focusing the

ways to optimize the spatial search strategies, Zhang et al. [127, 128] propose a multi-scale tumor modelling platform that understands brain cancer,

As concerning the basic structure and ingredients of a standard agent-based model built into the field of biology and medicine [125, 110, 129, 111, 130], the diverse types of agents, their behaviors (mechanisms of interaction), and their environment should be distinguished [103, 131] . All these aspects are discussed in the next section with reference to our model that, placing at a quite abstract level of simulation, models the interactions of a population of tumor cells with a population of peptides. Besides going deeply into the model structure, two general traits should be now highlighted. The first one is that it is based on the idea that cancer cells share with stem cells the capacity to replicate but not to differentiate: they are a kind of "faulty (mutated) stem cells". Hematopoietic stem cells have been already modelled [132], but here the emphasis is not given on the collective properties of "good" and proper stem cells, which produce their self-regulating behaviour. Rather, it is addressed the ways to induce their differentiation processes by means of "external agents" (the peptides), and the effectiveness of these processes under different circumstances related to the tumor growth rate and other parameters. The second peculiar trait is that this model focuses specifically on the spatial and temporal characteristics of stem cells differentiation processes. Hopefully, future research will merge the two modelling research streams, the one working on the collective properties of good and bad stem cells and this on the ways to induce differentiation processes into the bad ones.

5. An Agent-Based Model for Tumor Staminal Cell Differentiation

In this section, we engage in the construction of a quantitative model that brings together the result of the conceptualization of tumor cells behavior described in the previous sections. Our key proposition is that the tumor behavior and evolution can be described as the evolution of a system composed by two main categories of actors. Tumor cells (as mutated stem-cells) and factors (or peptides). Let us then examine the rules followed by each of these agents.

Tumor cells. According to the results of cancer research mentioned in Sections 2 and the conceptual model of Section 3, cancer cells can differentiate into a certain number of stages. In particular, according to [44], 5 stages. The stages are numbered from 0 to 4. The number is related to the type of cancer cell considered, and goes from most malignant cells [pluripotent] (0) to healthy cells. Therefore, 5 types of cell agents are present in the model: pluripotent [stage 0], multipotent [stage 1], oligopotent [stage 2], differentiating [stage 3], completely differentiated [healthy, stage 4]. Tumor cell agents of each type behave according to the following rule: it reproduces in two tumor cells of the same type, unless a positive interaction with a differentiation factor occurs. This rule follows the observation that, if a tumor is left uncured, it does not regress spontaneously. Conversely, if a positive interaction occurs, the tumor cell differentiates in a less malignant stage. At each step of the simulation cycle, every cell has a certain probability to duplicate itself. This probability is parameterized in our model by a number (Growth-Rate), which is characteristic of each differentiation stage (more malignant stages have a faster reproduction rate). In this way, we model the fact that cells do not reproduce with a deterministic frequency.

The second type of agents are differentiation factors, that when administered, make tumor cells differentiate into a less malignant stage. In principle, n_F types of factors can be considered (n_F is flexible). In our model, we have inserted 4 types of differentiation factors. This is enough to model the action of molecules that cause each tumor cell agent to move one step down in the malignancy scale. No factor is needed (or act) on healthy cells, therefore the types of factors is equal to the types of cells less one. The rule followed by factors are as follows. A factor can cause at most one differentiation during its active life; no differentiations can be caused after its active life. The characteristic features of differentiation factors are: a) their active life, b) their administration frequency, c) their administration doses and d) their fitness. This last feature is a fundamental parameter to describe the interaction among differentiation factors and tumor cells. The interaction is modelled as follows.

- Both tumor cells and differentiation factors are represented as interacting agents. These agents are free to move randomly (Brownian diffusion) in a common plane (see Fig.1). Diffusion speeds are here considered to be inversely proportional to the particle size (i.e. factors will move 1000 times faster than cells). In this way, at each step of the simulation, there is a certain probability that a tumor cell agent interacts with a differentiation factor agent, proportional to both factor doses and cell densities (Figure 1).

[Insert Figure 1 about here]

Whenever a cell meets a differentiation factor, it may undergo a differentiation process and move to the next stage, with a certain probability. This probability is, indeed, what we call fitness.

In general, fitness is described by 4x4 matching matrix A, with entry a_{ij} describing the probability that differentiation factor i induces a mutation in cancer cell of stage j, once they meet.

Let us then analyze the model from a more technical side, namely the "input" and "output" perspectives. The input data are the initial number of tumor cells, the frequency of factor administrations, the factors doses, the fitness matrix A, and the growth rates. The "output" data are the evolution of each type of agent with time.

The model presented here has been developed using NetLogo 4.04 environment. Figure 2 shows the result of a simulation in which cancer cells (stage 0) are not subject to any treatment.

[Insert Figure 2 about here]

From Figure 2, one notes that the model reproduces an exponential growth of cancer cells, in accordance with the rule (and intuition). Cancer cells grow exponentially, if they are not subject to a cure. This curve can be used to determine the values of the growth rates by fitting actual numbers obtained in an experiment, as we are to see. Just to investigate into the model behaviour, we present a second numerical experiment concerning the extreme case of factors having 100% probability of induce cell differentiation, in a diagonal fitness matrix. At the beginning of the simulation, only undifferentiated cells are present. All five types of factors are administrated with a frequency of

8hs. We obtain the result in Figure 3, where the number of cells at each differentiation stage is plotted as a function of time.

[*Insert Figure 3 about here*]

The results plotted in Figure 3 correspond to an hypothetical case and no experimental data are available to compare the result of the simulation. However it's interesting to observe the following features: a) the regular spikes in the right graphs and b) the growth in the different populations of cancer cells agents. In the left graph, the number of administrated factors is, at each administration, equal to the number of malignant cells. Let F_i denote the number of factors of type i at an administration. Then, it is $F_1=F_2=F_3=F_4=C_0$, where C_0 is the number of malignant cells at stage 0, present the beginning of the simulation. By the 100% fitness, interactions between cells and differentiation factors lead to an immediate decrease in the malignant cell population, We note that a pluripotent malignant cell of stage 0 when interacting with a factor is turned into a multiponent malignant cell of stage 1. In the left graph of Figure 2, one then notes the initial rapid growth of multipotent malignant cells (orange line), which then start declining due to the action of the differentiation factors, which are in number greater than the newly born multipotent malignant cells. Then, at a later stage of the simulation, cells of stage 2 (oligoponent) appear, with an initial increase followed by a rapid decrease. One notes the rapid increase of healthy cells. The right graph, instead, depicts the situation of maximum fitness but with differentiation factors administered in a number that is overall equal to the number of tumor cells: $C_0=F_1+F_2+F_3+F_4$. In this case, since differentiation factors that act on pluripotent malignant cells are not enough to reduce the number of tumor cells in stage 0 (the ratio is 1 to 4), tumor cells start growing when factors are consumed after the initial interactions (recall that there is a 100% fittness), until their growth is again counterbalanced by the new administration. The inception of the different tumor cell populations follows a delay similar to the one of the previous case. However, one notes that, since stage 1 is generated by stage 0, when stage 1 is increasing, stage 0 is decreasing, and conversely.

Let us then come to input parameter fitting based on actual experiments. One can split these parameters into two sets: a) parameters related to the biological properties, and b) choice variables. By biological parameters, we deem all those input data that are not under the control of the decision-maker (physician). As we discussed in detail in Section 4, these parameters need to be estimated by experimental data and literature data, to obtain reliable predictions. Choice parameters, instead, are the parameters that can be controlled by the physician. These parameters can be chosen by the physician so that to obtain optimal results from the cure. In our case, these parameters are the doses and administration frequency of the differentiation factors.

Let us analyze first the fitting of biological parameters. Cell growth-rates, densities of cells and differentiation factors must be regulated for the specific cancer cell lines considered. Growth rates for cancer cells were derived from empirical data in [39]. To estimate the fitness parameters, we matched our simulations to experimental data from in-vitro experiments [39] in which 5 different cancer cell lines are treated using 3 differentiation factors obtained from Zebrafish embryo. Using the least square methods we calculated the fittness parameters. We performed simulations using 1000 initial cells and 250 factors, obtaining the following probabilities (fitness):

a) 4.0% for the interactions of differentiation factors of type 1 with pluripotent malignant cells;

b) 5% for the interactions of differentiation factors of type 2 with multipotent malignant cells;

c) 2.5% for the interactions of differentiation factors of type 3 with oligopotent malignant cells;

d) 1% for the interactions of differentiation factors of type 1 with differentiating malignant cells;

The last stage should is the “healthy cell” stage and it’s growth rate is therefore irrelevant to the purpose of our simulations. We therefore obtain a diagonal fitness matrix.

By the above data obtained from experiments and literature data, it is then possible to perform virtual experiments to analyze different selections of the choice variables. In particular, we have carried out several experiments by varying the differentiation factor dose, administration frequency, and time horizon. In the next paragraphs we present the most significant results, with the purpose of highlighting the insights that can be derived from the model.

Let us start with a simulation in which we have the initial conditions as in the simulations of Figure 3, but in which the actual values of the fitness as matched by the numerical experiments is used. Thus, the only difference between the results in Figure 3 and the next Figure 4 is represented by a variation in the matching probabilities.

[Insert Figure 4 about here]

The right graph in Figure 4 shows that, in the case $C_0=F_1+F_2+F_3+F_4$, differentiation factors are capable of reducing the presence of cells of type 0 of around 40%. Their main effect is, then, a reduction in the tumor progression speed. However, they are not in sufficient number to generate a significant number of healthy cells. One could think that this effects is to be attributed to the time scale (only two days). We then performed additional simulation experiments with a longer time horizon. Results confirm the slowed growth of tumor cells, however lock-in is not reached.

The result of Figure 4 (right graph), then say the following to a physician: either the concentration of the frequency of factor administration is not sufficient to contrast the tumor expansion.

Let us then hypothesize that the physician decides to act on factor concentration. Using the condition $F_1=F_2=F_3=F_4=C_0$, which is equivalent to multiplying each factor' concentration by 4, one obtains the results in Figure 4 right. One notes that the concentration ratio is now such that a decrease in the tumor cell polulation is achieved.

Alternatively, one can act on administration frequency. By administering the vaccines every two hours, instead then every 8hours, one obtains the result reported in Figure 5. Results are comparable

to the effect of administering a higher concentration at a lower frequency (one can compare the left graph in Figure 4 to the results of Figure 5).

We performed also a series of virtual experiments to analyze the behaviour of the populations as the concentration of factors raises. Figure 6 reports results for the case in which the following concentration ratio is used: $F_1+F_2+F_3+F_4=4C_0$, namely, all factors are in a number which is 4 times higher than the initial population of tumor cells. Results show a notable improvement in the tumor evolution (the time scale is also longer, namely 4 times longer than in the previous simulations.)

[Insert Figure 6 about here]

6. Conclusions

Recent discoveries in tumor research have lead to the identification of the central role played by the differentiation process of cancer cells-like stem cells. This has lead scientists [44] to conceive a new medical model of the desease.

Purpose of this work has been to translate this new concept into a quantitative model capable of simulating its evolution as per the recent paradigm. We have seen that the agent-based methodology (ABM) is the adequate tool for accomplishing, because of the shift implied by the new paradigm from a reductionists approach to the theory of complexity and of the link between complexity theory and the AMB methodology.

We have then realized a model that foresees two main types of agents: cancer cells-like stem cells, and differentiation factors. In particular, 5 types of cancer cells like-stem cells agents, and 4 types of differentiation factors, in agreement with the differentiation stages from most malignant to healthy. Differentiation factors interact with cancer cells, and if a positive interaction occurs, cancer cells are caused to differentiation in the next stage of lower malignancy.

By distinguishing between biological parameters and choice parameters, we have seen which input data need to be fitted from experiments and which parameters are, instead, in the hands of the physician. It is on these parameters that the decision-maker can act to understand how different

doses and frequencies of differentiation factor administrations can lead to an improvement in the disease evolution.

The availability of the simulation tool has the advantage that insights on trends and on the significance of the choice variables can be obtained by performing virtual, instead of real experiments.

This work paves the way to further research. The simulation model results can be used, when compared to experiments, to prioritize which parts of the models (both the conceptual and simulation ones) need to be further studied in order to improve our understanding of the disease mechanisms. As an example, factors are currently administered all at the same time. The model can then help in the identification of which factor is most effective to induce differentiation at a given stage and in a given tumor type. This aspect constitutes the first step in future research, and is part of ongoing research by some of the authors of this work.

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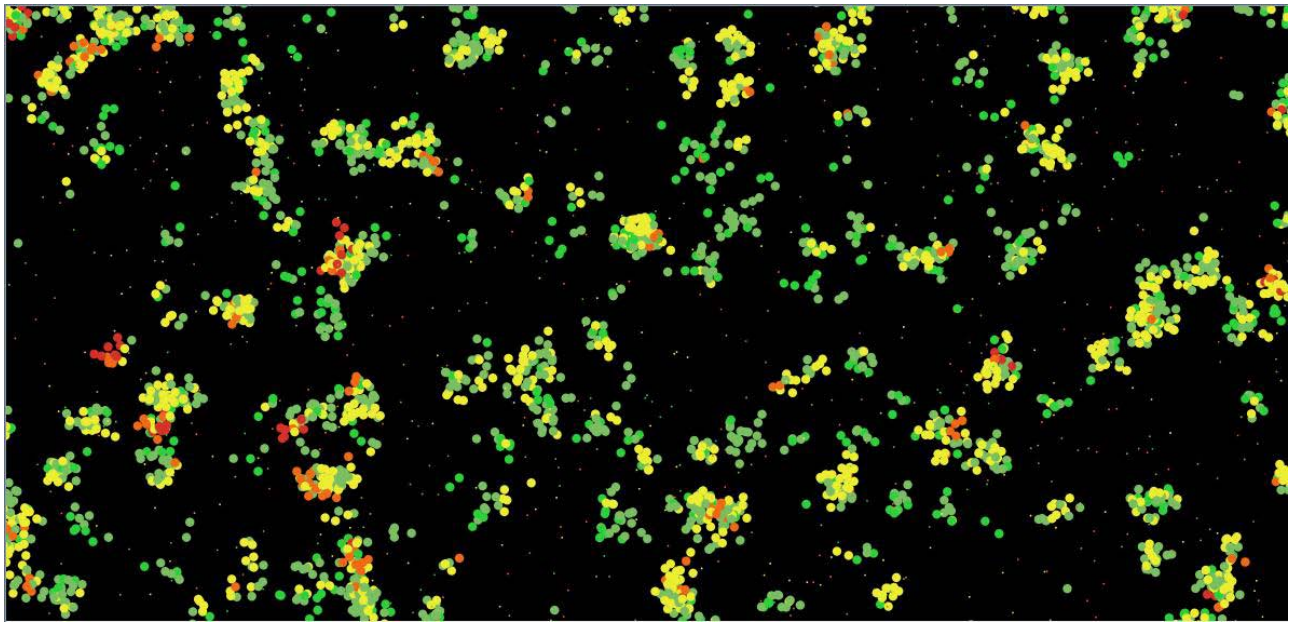

Figure 1: *Both cells (big dots) and differentiation factors (small dots) diffuse freely in a common plane. The chances that a cell meets the appropriate differentiation factor are therefore related to the respective densities. Different colors of agents refer to different differentiation stages.*

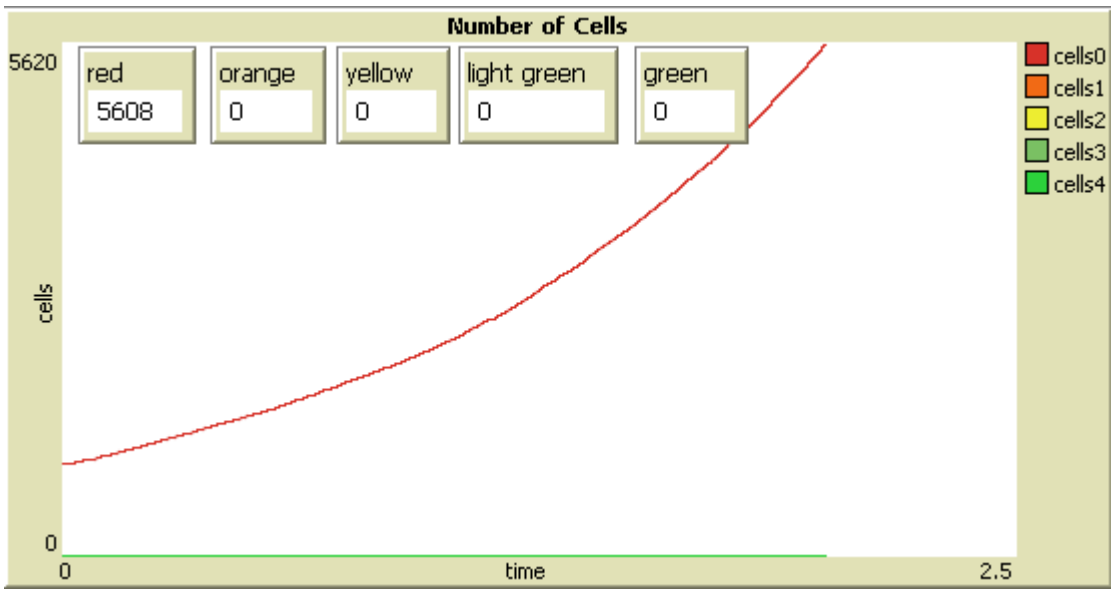

Figure 2: *result of a simulation in which cancer cells are not subject to any treatment. Red: Stage 0, Orange=Stage 1, Yellow=Stage 2, Light Green=Stage 4, Green=Healty Cells*

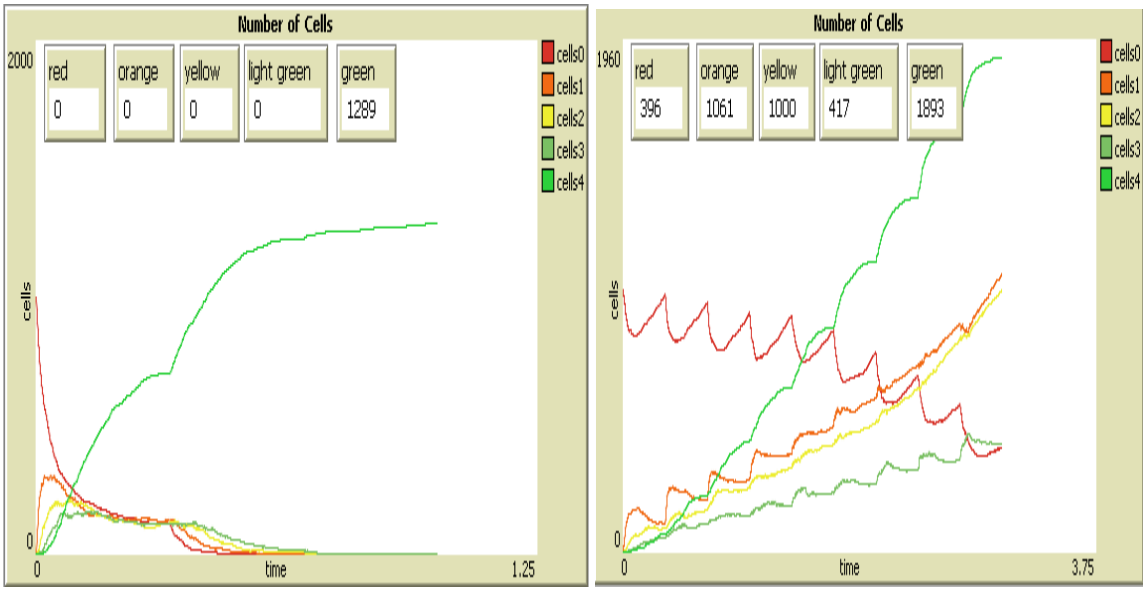

Figure 3: *Simulation results for the hypothetical case of 100% factor fitness. In the left graph, we have* $F_1=F_2=F_3=F_4=C_0$*; in the right graph, we have* $C_0=F_1+F_2+F_3+F_4$*. Legend: Red=Stage 0, Orange=Stage 1, Yellow=Stage 2, Light Green=Stage 4, Green=Healthy Cells*

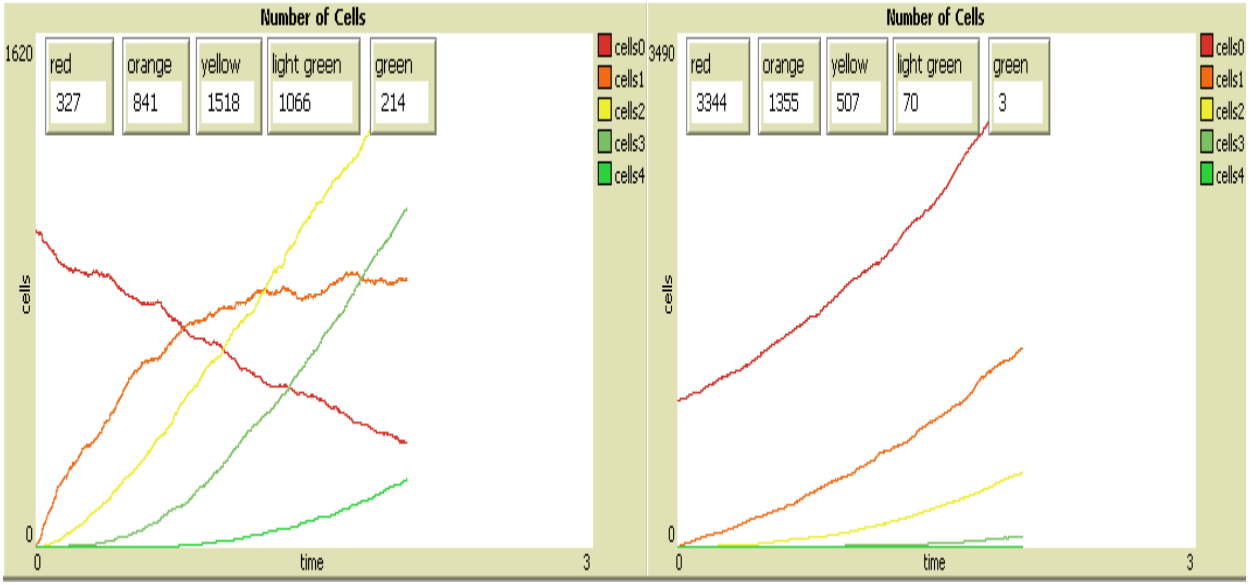

Figure 4: *Simulation results with fitness matched from experimental data. In the left graph, we have* $F_1=F_2=F_3=F_4=C_0$*; in the right graph, we have* $C_0=F_1+F_2+F_3+F_4$*. Legend: Red=Stage 0, Orange=Stage 1, Yellow=Stage 2, Light Green=Stage 4, Green=Healthy Cells.*

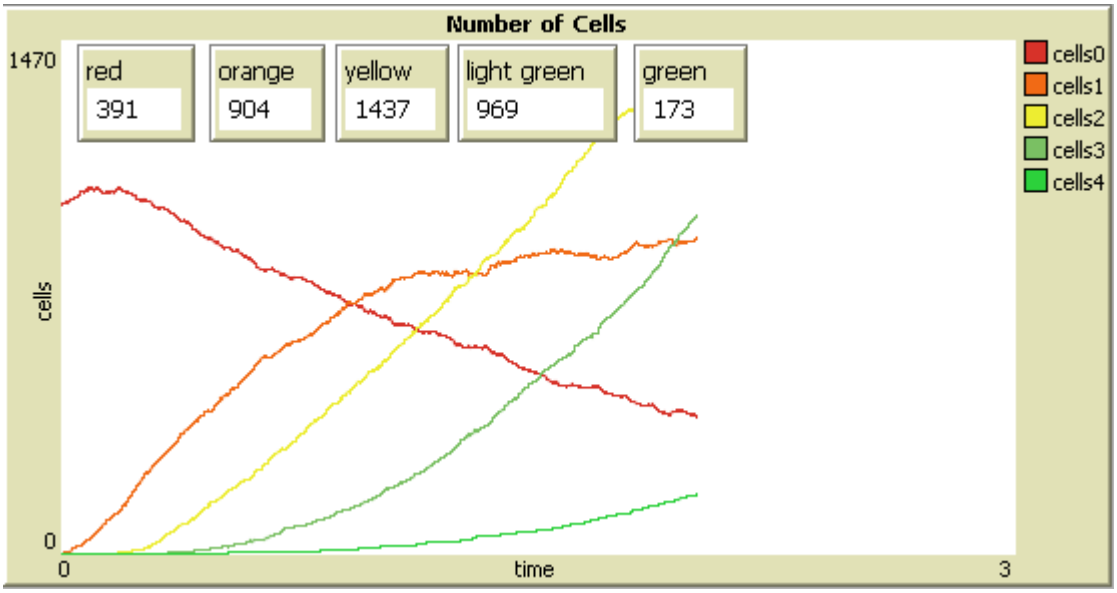

Figure 5: *Effect of increasing the administration frequency. while leaving $C_0=F_1+F_2+F_3+F_4$. Legend: Red=Stage 0, Orange=Stage 1, Yellow=Stage 2, Light Green=Stage 4, Green=Healthy Cells*

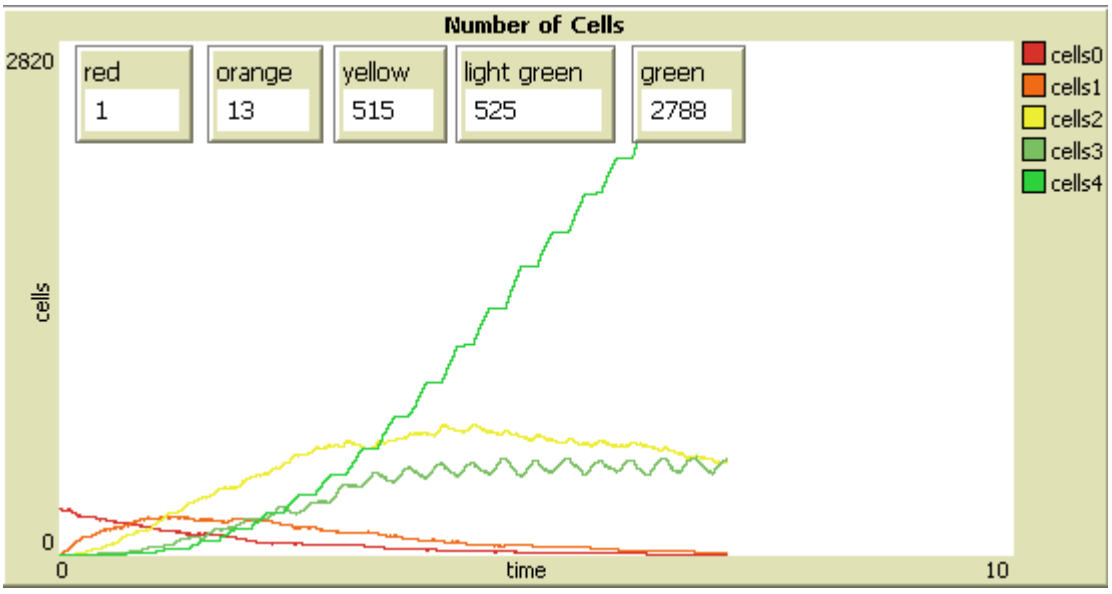

Figure 6: *Effect of increasing factor concentration to 4 times the concentration of tumor cells. $4C_0=F_1+F_2+F_3+F_4$. Legend: Red=Stage 0, Orange=Stage 1, Yellow=Stage 2, Light Green=Stage 4, Green=Healthy Cells*