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MATHEMATICAL MODELS OF STEM CELL BEHAVIOR (M KOHANDEL, SECTION EDITOR)

Integrating Biological and Mathematical Models to Explain and Overcome Drug Resistance in Cancer, Part 2: from Theoretical Biology to Mathematical Models

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Abstract
Purpose of Review Despite the promise of targeted cancer therapy (i.e., drugs targeted towards specific signaling pathways supposed to be essential to the survival and proliferation of cancer cells), unexpected treatment failures in the clinic are common. Tumor cell heterogeneity, which can result from the plasticity of individual cancer cells and/or evolutionary dynamics, has revealed the importance to study cancer at the level of the entire cell population. Here, we explore mathematical models that describe tumor dynamics under pressure of anticancer drugs by integrating cancer cell population heterogeneity and evolutionary behavior. We further explore mathematical modeling as a theoretical tool to analyze and predict the behavior of cancer cell populations as a whole, and not only of individual cells, which may reveal new clues to therapy failure and ways to overcome it.
Recent Findings An evolutionary perspective that relies on the "atavistic theory of cancer" together with the so-called "cold genes", and the involvement of "bet hedging" in tumors, has recently changed our vision of cell plasticity in cancer. These new perspectives provide a sound theoretical basis to the emergence of resistance in cancer cell populations and to its possible reversibility. Continuous mathematical models of the evolutionary dynamics of proliferative cell populations already exist, that take into account the heterogeneity of cancer cell populations, allowing to study the evolutionary potential of cancer cell populations and predict their behavior. Those models, in turn, can be used to probe population growth control by incorporating functions (in the mathematical model) that represent the action of drugs on the mechanisms driving proliferation, and furthermore to suggest new therapeutic strategies in the clinic of cancers.
Summary In this second part of our review, that can be taken independently of Part I, we focus on the level of cell populations, the only one amenable to completely take into account phenotype plasticity in its observable consequences on the evolution of proliferative diseases, and on heterogeneity, that makes sense only in the context of cell populations, together with the fundamental evolutionary potential of cancer cell populations (and not only of single cancer cells). These are modern views that come from the transposition from ecology of species to the biology of cancer (for further reading on evolution and cancer, the reader is referred to the recent comprehensive book on the subject Ujvari et al. 2017). Finally, we present a brief review of mathematical models, with their features and their use in cell population studies, to

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account for phenomena found in cancer, focusing on drug resistance. We contend that a good understanding of what mathematical models can do to tackle the question of drug resistance in cancer can shed light on the mechanisms of resistance and means to control them and help design principles for biological experiments to be performed at the lab and therapeutical strategies to be applied in the clinic of cancers.

Keywords Cancer · Therapy resistance · Cell populations · Evolution · Biological models · Mathematical models

Introduction: Cancer Cell Populations, Not Only Single Cells, to Study Drug Resistance

Insufficiency of Single-Cell Level Studies That Assume Heterogeneity of Cancer Cell Populations

Why is it that such promising molecules used in targeted therapies, such as the tyrosine kinase inhibitors or monoclonal antibodies, often work quite well in a tissue culture dish, i.e., usually on isolated homogeneous cancer cell populations, but in human organisms show only transient effects [2, 3]? Notwithstanding notable progress elicited by imatinib [4] and all-trans retinoic acid [5] in some hematological malignancies due to chromosomal translocations (CML and APL, respectively, and in some other tumors, like GISTs, for imatinib), these very special cases in which the disease is intrinsically linked with an isolated molecular event do not easily generalize (let alone the fact that resistance to imatinib may also occur [6]). Different reasons for the failure of such promising therapeutics have been proposed (see Part I and [2, 7]), but a highly likely cause is the extended heterogeneity, with respect to the expression of resistance genes, of the population of cancer cells in a given tumor. Indeed, assuming such heterogeneity, even if an overwhelming proportion of cancer cells in the tumor are killed by a drug, it suffices that a tiny subpopulation survives to allow the tumor to resist and eventually thrive again. Such phenotypic heterogeneity, that need not be due to mutations, may be spatially distributed or not, may be due to the selection of preexisting specialized cells, or to mere continuous epigenetic adaptation to drug insult (discussed in [8]). Independently of this alternative between selection and adaptation is resistance due to a single multiresistant clone or to stochastic parsimonious risk spreading of specialized resistance phenotypes in the cancer cell population? The latter case is a would-be "tumor strategy" known as *tumor bet hedging* (see below). A full explanation of failures in cancer therapeutics, therefore, has to deal with various types of heterogeneity in cancer cell populations, forsaking the paradigm of the single resistant clone.

Plasticity of Cancer Cells: from the Single-Cell Level to the Cell Population Level

Plasticity (see Part I) may be defined as the ability of partly mature cells to dedifferentiate and possibly later redifferentiate to adopt another phenotype. This ability is generally attributed to cells showing a "stem-like" status. However, it must be stressed that "stemness" is not a phenotype in itself, but rather a transient cell state that cells may acquire and lose [9, 10]. Such labile transitions are of epigenetic nature, due to DNA methylation, acetylation, or methylation of histones [11] mentioned in Part I. The epigenetic enzymes involved can be used by dedifferentiated cells to yield drug resistance. For instance, the histone lysine demethylase KDM5A has been found to be responsible for acute drug resistance in an aggressive line of lung cancer (non-small-cell lung cancer, NSCLC) cells [12]. The genetic potential for phenotype change, leading to adapted partial dedifferentiations, on which epigenetic control may act, is present, masked in our genome by epigenetic barriers established in the course of our development towards multicellularity to design coherent organisms based on cooperation between differentiated tissues [13]. Cancer cells, already partially dedifferentiated or improperly differentiated, are particularly fit to divert such epigenetic enzymatic mechanisms (KDM5A mentioned above [12], DNMT3A [14] in leukemogenesis leading to acute myeloid leukemia, and others) for their own adaptive benefit.

Survival of a tumor in an environment changing to extremely hostile conditions must be considered, as mentioned above, in the cancer cell population as a whole, and it cannot be considered independently of the dimension of time. What would be the value of an adaptive mechanism if it were too slow to counter a fast life-threatening insult coming from the environment? In this respect, dynamic biological experiments must be performed, such as the one reported by Sharma et al. [12]. Instead of studying resistance in cancer cell populations using a slow gradual increase of drug doses, as has been typical in many studies, in order to yield resistant cell lines, they exposed an aggressive lung cancer cell line to very high doses (about 50-fold higher than the dose used in the clinic) of drugs, to study the adaptive mechanisms at stake. The "dynamic" aspect of their study was that they continuously followed over time the gene expression pattern in the surviving cell population. For instance, what is the initial status of methylation of genes of interest in cancer cells? What are the dynamics of critical enzymes involved, such as demethylases and methyltransferases [11]? It should be possible, using such dynamic studies, to precisely define plasticity as a velocity of adaptation to diverse environmental changes. Ideally, to avoid averaging effects when they are performed in whole cell populations [15], such dynamic gene expression studies should be performed at the level of single cells, *for many different cells in the same cell population*, focusing on epigenetic enzymes

that underlie plasticity, to explore such plasticity in a heterogeneous cell population by reconstruction of the distribution of gene expression by sampling each cell in the total population.

Phenotype and/or Spatial Heterogeneity Accounts for Evolution Towards Resistance

Genetic driver mutations can give rise to resistance and have, for many years, been considered the only cause of resistance in cancer. This may often be the case, as exemplified and illustrated previously [2]. However, resistance also occurs without mutations, for it may be totally reversible, as shown in cell line studies performed by Sharma et al. [12], if one waits for enough time after the drug has been washed out from the culture dish. In this completely reversible case is resistance of sheer epigenetic nature, or is reversion to sensitivity due to the quasi-extinction, when the drug has been washed out of a resistant, mutated clone that was initially present in a dormant state and was selected by exposure to the drug? This has been discussed, e.g., in [8, 16, 17], opposing a Lamarckian-like adaptive behavior of the cell population to a more classical Darwinian vision, relying on sheer selection of preexisting resistant cells. Whatever the explanation, it must assume phenotype heterogeneity in the cell population, with evolution from total drug sensitivity to total resistance—and possibly back. At the cell population level, through a varying percentage of expression of resistance genes, as at the single-cell level, through, e.g., variable methylation status, a resistance phenotype continuum is most likely, as opposed to the naïve vision of a binary totally sensitive vs. totally resistant status of cells or subpopulations in a tumor. The likelihood of such a continuum allows for continuous evolution in a resistance phenotype rather than binary switching between total sensitivity and total resistance to a given drug. Heterogeneity may be considered in a first approach only of spatial nature, obviously more easily studied by using spatially isolated samples in the same tumor and its metastases, with phylogenetic reconstruction by branching processes [18]. However, it can also be considered as merely phenotypic, independently of space, with possible spatial coexistence of reciprocally tolerant, possibly symbiotic, clones in the tumor [19, 20].

Stochastic *Bet Hedging* in the Expression of Genes in Cancer Cell Populations?

The concept of *bet hedging* in tumors, of course, initially came from the world of economics, in which it was long ago theorized as the best way to preserve one's assets, but it has also been present in evolutionary ecology, e.g., [21, 22], and microbiology, e.g., [23], usually related to hedging in time (in particular, of reproduction) or space. It may be summed up in the case of a species or of a population of individuals exposed to changing environmental conditions as different choices in the same population for essential fates regarding the survival of the species or population. For a population exposed to a changing environment, is it a winning strategy to endow only one of its parts with all the power to face adverse events, or is it better to give different tools or weapons to different parts of its population, with the hope that, in the case of a confrontation with an unexpected life-threatening catastrophe, at least one part will be able to survive and preserve the population? Insofar as such a successful strategy is the result, not of a mutation, but of a non-genetic change of phenotype, when the life-threatening danger is over, the whole population may be totally preserved as such, since part of it has survived and can thrive again.

References on bet hedging in cancer are not as numerous as in ecology or microbiology. One can cite (Brutovsky & Horvath, arXiv 2013 1307.060; [24, 25]), studies in which the case of cancer cell populations is considered. In cancer, as opposed to the classical view of one single clone that becomes more and more dedifferentiated and acquires pluripotent drug resistance (MDR) due to a single renegade cell, a so-called cancer stem cell, the bet hedging hypothesis assumes parsimonious distribution of specific resistant phenotypes in the cell population. How can such a risk-spreading strategy be achieved? One may speculate that it can be seen as the diversified, likely stochastic, result of the (epigenetic) unmasking, triggered by abrupt changes in the environment, such as cytotoxic drug pressure, of so-called "cold genes" [26], in the whole population. Such "cold genes," that are highly preserved throughout the general evolution of multicellular organisms (by contrast to "hot genes" that carry all of the evolvability of the genome), could be responsible for simultaneously, and likely also stochastically, launching reversible stress responses of diverse natures that have been stored in the genome in the course of billion-year-long evolution from the first unicellular organisms to face life-threatening events such as UV radiation, acidity, hypoxia, or other cellular stresses [13].

Evolutionary Phenomena in Cancer Cell Populations

"Nothing in biology makes sense except in the light of evolution" (Theodosius Dobzhansky [27]).

Evolution of Multicellular Life and the Atavistic Hypothesis of Cancer: the Large-Time Scale

The *atavistic hypothesis of cancer*, recently popularized in [13], is not a new idea [28]. It simply states that cancer may be seen as a reverse track in the great, billion-year-long, evolution that has led to the present most evolved multicellular organisms, and that genes that are altered in cancer are those which precisely correspond to the main steps of

multicellularity construction through a "multicellularity gene toolkit" [29, 30]. As evolution proceeds by tinkering [31], likely some of these tinkered steps driven by genetic mutations show flaws and are sensitive to environmental changes, in some differentiated tissues of (likely) all individuals. Searching systematically through the fragile steps in the development of multicellularity, which is initiated by proliferation control, followed by control of differentiation, with epigenetic controls coming last and being the most delicate, and comparing them with cancer phylogenetic studies should help rationalize genetic studies in cancer. Such vision might yield probabilistic study models of fragility in the genome and help design targets in models to focus on in therapeutic interventions. Independently of such potential studies, it should be stressed that we bear in our genome various defense mechanisms (against hypoxia, UV radiation, acidity, etc.) coming from our ancestors in a remote hostile past of the Earth [13], that have normally been epigenetically silenced to allow for a coherent organization of cooperating differentiated cells and tissues in multicellular organisms, but that can be unmasked in the very plastic cancer cells. This may account for the fact that poorly differentiated cancer cells can develop many resistance mechanisms, as outlined in, e.g., [32].

Ecological Evolutionary Mechanisms in Cancer Cell Populations: the Short-Time Scale

On the short-time scale of evolution of cell phenotypes in a given genome in a human life (and not of all genomes in the changing environment of the planet Earth, as in the previous case), cancer cell populations may be considered as new species that aim at thriving at the expense of other surrounding cell populations that exist within a multicellular environment. Such ecological views applied to cancer, often qualified as cell Darwinism, lead one to consider the evolution of cell populations in their environment, which is constituted of exchanges with other cell populations, in competition or symbiosis, of local metabolic conditions such as oxygen, pH, glucose, growth factors, inflammatory cytokines [33], and also of local mechanical tension/pressure in a given tissue. This short-time scale of observation is easier to take into account by observations than large-time phylogeny. It is the first setting in which to develop models of non-genetic adaptation [8]. It can also involve phylogenetic studies of successive clonal mutations in rapidly evolving diseases that help understand their history, as in [34, 35]. Of note, in this latter study [35] dedicated to the analysis of blood samples of patients with acute myeloid leukemia, the prevailing functional and phylogenetic scenario consists of a first mutation in an epigenetic control gene, followed by a mutation in a transcription factor related to differentiation, and finally by a mutation in a gene related to proliferation, as if it recapitulated a systematic deconstruction of coherent evolution towards multicellularity [13].

Evolution Towards Resistance: Irreversible (Due to Mutations) or Not (of Epigenetic Nature)

The introduction of an evolutionary point of view in cancer owes a great deal to studies by Gatenby et al. [36–38], see also [1]. The distinction between mutational (genetic, irreversible) and non-mutational (phenotypic, often of epigenetic nature, reversible [16, 17]) mechanisms has already been mentioned above in the context of heterogeneity in cancer cell populations characterized in their biological diversity by variable expression of drug resistance. It should be noted that genetic and non-genetic mechanisms are not mutually exclusive. In an evolutionary perspective, the dominant opinion is that mutations come first. However, the reverse has been observed [39] and a succession of epigenetic/genetic/epigenetic changes is completely possible [40]. Nevertheless, when reversibility of resistance is obvious from experimental observations (although one can never completely exclude the emergence of a resistant mutated clone followed by its extinction), it is tempting to involve epigenetic control, all the more so when epigenetic enzymes have been identified, as in [12]. Whatever the underlying mechanisms, mathematical models can capture such reversibility and can be used to design optimized therapeutic drug regimens overcoming drug resistance (see below).

Mathematical Models: Their Features for the Study of Evolving Cancer Cell Populations (See also [41] for equations and illustrations)

Probabilistic Models with Discrete Time (Number of Divisions) or with Continuous Time

The first probabilistic model that takes drug resistance into account was proposed in 1979 by Goldie and Coldman [42]. They assumed that there is a certain probability of a mutation (to gain the resistance phenotype) with each cell division. This model was extended by Goldie et al. [43] to study drug resistance to two different types of chemotherapeutic agents. Indeed, long-term patient survival not only depends on minimizing the total number of tumor cells but also depends on preventing the development or outgrowth of drug-resistant subpopulations within the tumor. As regards the dynamics of stem cells without drugs, Pacheco and Dingli have also proposed purely probabilistic models [44, 45], in which time is discrete and consists of numbers of divisions at mitosis. They argued that in the case of stem cell populations, cell numbers are too small to use continuous equations (that represent the evolution of large population numbers). They applied their methods to the hematopoietic maturation tree, using allometric scaling in mammals to reconstitute it in humans [46]. Multitype continuous time branching processes have been used to study evolution of

resistance mutations emerging from an exponentially growing population of cancer cells [32, 47].

Agent-Based Models: Simulations That Easily Take Biophysical Phenomena into Account

Agent-based models (ABMs) do not involve continuous nor deterministic settings. They are by nature based on stochastic evolution rules according to which cells (agents) divide, move, differentiate, or die, with a priori total unpredictability. As no mathematical analysis (i.e., no theorems and thus no sure predictions) can be obtained from them, they can be used only to perform simulations up to a limited number of cells, usually around 10,000, due to their heavy computational burden, without any certainty that their results are always reproducible, and not simply due to random effects. However, any metabolic or environmental influence exerted on the cells, provided that it has a chemical or physical representation, can readily be included in such models. Furthermore, these models can provide biologists and mathematicians, concerned with sure predictions about the fate of a cell population, with conjectures, i.e., with an intuition of what could be proved. For these reasons, they are popular in the community of mathematical modelers, even among those who indulge more in PDE modeling.

For a comparison between partial differential equations (PDEs, see below) and ABMs, see [48]. Roeder and Loeffler [49] have used ABMs to dynamically study imatinib-treated chronic myeloid leukemia. See, however, below about partial differential equations (PDEs, Kim and Doumic in a simpler setting [50, 51]). Gupta et al. [52] have used ABMs to simulate stability of phenotype coexistence in cancer cell populations, and later Chisholm et al. [8] have also used ABMs to investigate stochasticity in the evolution towards drug resistance and its reversibility, as biologically evidenced by Sharma [12]. Poleszczuk and Enderling also used an ABM of cancer stem cells and their progeny, including transition between stem and non-stem cancer cell states, and showed that plasticity can substantially increase tumor growth rate and invasion [53].

Ordinary Differential Equations: Compartmental, Simple, Amenable to Dynamic Analyses

On the contrary, ordinary differential equations (ODEs) are, by nature, continuous in time, deterministic, and can give rise to theorems and predictions of the fates of cell populations. Note, however, that they are valid only in the case of large population numbers (which of course may be questionable in the case of healthy stem cells). They represent the necessary fate of a cell population, provided that one knows its initial conditions and dynamic rules for its instantaneous evolution, which can be described by conservation laws such as the law of mass action. Consideration of heterogeneity in ODE models is, however, limited to a discrete representation, i.e., residing in a compartmentalization between totally homogeneous subpopulations and exchanges of cells between these compartments. This is exemplified in [54], in which an additional environmental framework is proposed.

A variety of ODE models have been developed to study the dynamics of cancer stem cells, heterogeneity and plasticity of cancer cells, and the role of stem cells in drug resistance. Several studies have focused on mathematical modeling of stem cells and cancer initiation and progression in chronic myeloid leukemia (CML), see, e.g., [55–57].

By calculating the probability of resistance, Komarova and Wodarz [58] developed an ODE model for the targeted treatment of cancer based on the evolution of resistant tumor cells. Their model suggests that resistance often arises before the start of treatment. The cancer stem cell hypothesis has also been applied in the context of drug resistance as an evolutionary process by Leder et al. [59], of course still in a binary (compartmental) vision, since ODEs are not amenable to take continuous phenotypes nor continuously evolving transient cell states into account. The model by Leder et al. suggests that dedifferentiation reduces the effectiveness of therapy directed at cancer stem cells by increasing the rates of resistance.

Age and Maturation-Structured Transport PDE Models and Delay Differential-Derived Models

Age-structured models have been used to represent the cell division cycle in cell populations. In this case, heterogeneity occurs only with respect to an age variable [60–63]. More PDE models, structured in both age in the cell cycle and maturation (=differentiation) age, either with continuous [64] or discrete maturation age [65], have been designed to simultaneously represent proliferation and maturation, in particular for hematopoiesis, from the hematopoietic stem cell to a given mature lineage such as neutrophils in the myeloid case. In the context of cancer evolution, plasticity and dedifferentiation are explored using an age-structured PDE with diffusion—to account for the probability of fixation of a mutation derived from a Moran process—in [66], with good fit to data. In this version of PDEs, the phenotype represented is only maturation, named age. Age-structured linear PDEs can immediately be reduced to delay differential equations that are not necessarily simpler to study, but simpler to formulate, which has been done in [67, 68]. A feature of such delay models is that they are amenable to represent periodic hematological diseases, using a delay systems version of a Hopf bifurcation. They are still a subject of active research, reviewed in [69].

Spatiallly Structured and/or Phenotype-Structured PDE Models

Phenotype-structured populations take into account the biological variability at stake by means of a continuous phenotypic trait characterizing cells in the population. These cell population models may sometimes be too complex to perform mathematical analyses, allowing only computer simulations, but they have the essential property of representing phenotype heterogeneity *in a continuous and reversible way*. This latter feature has been used, for instance, in [8] to question, in a phenotype-structured PDE model setting, the respective roles of non-genetic instability and of fast drug dose-dependent, adaptation in the establishment of acute drug resistance in cancer; a numerical study of a more general class of models is also performed in [70]. Classical PDEs usually take account of heterogeneity in cell populations in a spatial manner, i.e., the distribution of cells in a population and its evolution with time is dependent on the position of a cell in the spatial structure in which it is located. This allows, in particular, representing and analyzing cell motion triggered by chemoattractants, by means of the well-known Keller-Segel model [71]. This setting also allows one to study tumor growth in spheroids and its control by drugs, with possible application to drug resistance, as in [72], and many others. Structuring a cell population according to its heterogeneity in phenotype is less classical than with respect to space in cancer, although it had already been done in the framework of modeling for ecology, giving rise to the mathematical field of "adaptive dynamics." Such modeling has been proposed to account for a drug resistance phenotype in cancer in [73], where effects of therapies are studied and a proof of concept of drug delivery optimization is simulated, avoiding resistance and eradicating a cancer cell population, while preserving a healthy cell population. Furthermore, it is possible to mix phenotype and space when knowledge about the tumor geometry is known, such as in a spheroid, again to represent drug resistance and propose therapeutic strategies to overcome it [74].

Asymptotic analysis of an ODE or PDE model consists of determining, as a function of its parameters, its behavior in large time (otherwise said, for time tending to infinity). If the model represents a cell population, will its number stabilize at a given positive value? Will it become extinct? Will it grow indefinitely? Will it present sustained oscillations? In the case of several populations, will there be coexistence? At determined positive values? Or coexistent indefinite growth? These are the types of questions with which asymptotic analysis deals. Clearly, it allows for predictions, in the absence or presence of drugs that change the model parameters. This question is treated in an ODE setting, with possible applications to understanding and overcoming drug resistance, e.g., in [75], and lately in a phenotype-structured PDE setting for drug resistance [76].

It is sometimes possible to deduce a PDE model from an agent-based one, keeping its main features and taking advantage of the velocity of computation and of the possibility to perform some mathematical analysis. Such an example is provided by the Roeder ABM model of CML [49], and considerably reduced in its PDE version by Kim [50], and further analyzed for its stability by him and others in [51], obtaining the same results and more in terms of analysis. Nevertheless, it should be noted that, while the ABM formalism is easily accessible to elementary understanding, its PDE counterpart may seem more abstract, and even though it contains the same features and allows one to perform predictions that are certain (theorems are not subject to stochastic fluctuations), PDE models have too often been disregarded thus far—a trend that will hopefully be reverted in the forthcoming years.

Phenotype-structured models are also amenable to take evolution under metabolic change into account. This has often been studied in hybrid models, in which agent-based models represent the fate of a cell population, or several cell populations in competition, and the dynamics of diffusible molecules of interest in the tumor environment is represented by spatial PDEs of the reaction-diffusion type. This is the case, in particular, of the study [77], in which heterogeneity in cells is focused – in a continuous manner – on the oxidative vs. glycolytic metabolism, whereas proliferation and cell death are based on an ABM. Nevertheless, nothing opposes using strictly continuous (PDE) models to take environmental variables into account. This is done in a schematic way for nutrients and drugs in [74], with study of optimized therapeutic protocols.

Using Mathematical Models to Design Therapeutic Strategies Taking Drug Resistance into Account

As mentioned at the beginning of the previous section, structuring an evolving cell population according to a continuous variable x means assigning to each cell a relevant value x (or set of values if x is multidimensional) to characterize its variability at stake in the population. The variable x may be for instance age in a phase of the cell cycle, size, or expression of phenotype(s) of interest. Now, given a continuous and deterministic representation of the evolution of a cell population structured in phenotypes of resistance for both cancer and healthy cells in interaction by competition, e.g., for space occupation and for nutrients, one can set an optimal control strategy problem. Solving an optimal control problem, which is always finding a best trade-off between reaching an objective and respecting constraints inherent to the problem, here consists of determining the best strategy of continuous drug infusion that will contain, and if possible eradicate, a cancer cell population under the constraints of limiting unwanted side effects in the healthy cell population, while avoiding the emergence of a resistant phenotype in the cancer cell population.

This was, for instance, the case of the first models, consisting of compartmental ODEs, in which drug resistance in cancer and its optimal control by chemotherapy were studied [78, 79]. The framework of ODE models has more recently been used by Komarova et al. [58], Leder et al. [59] as mentioned above, taking drug resistance into account.

Adaptive therapy, as advocated by Gatenby et al. [36–38], brings the important consideration of an evolutionary drug resistance phenotype in the cancer cell population to the foreground. Either using ABMs or an ODE setting, or both, they develop the idea of making a binary (or possibly graduated) distinction between sensitive and resistant cancer cell populations and making use of this distinction in therapies. It amounts to taking the sensitive cancer cells as an ally inside the tumor fortress, since sensitive cancer cells are also the faster to proliferate in the absence of drugs. The trade-off consists in alternating periods of drug delivery, that kill sensitive cells but inevitably trigger proliferation in resistant cells [17], and periods of "drug vacation" during which, inside the tumor, resistant cells give way to the then more proliferative sensitive cells, the switch being determined by a threshold imposed on the tumor volume or mass.

Although not taking drug resistance into account, notable contribution has been brought to the field, still with ODEs, by Łędżewicz and Schättler, to optimize the delivery of a combination of cytotoxic and antiangiogenic drugs [80, 81], using optimal control algorithms. However, also using optimal control algorithms, and a combination of cytotoxic and cytostatic drugs in a phenotype-structured PDE setting involving reversible phenotype evolution, the question of simultaneously avoiding drug resistance and unwanted toxic side effects to healthy cell populations has been theoretically solved in the case of a finite horizon [76]. As in the case of adaptive therapy [36–38], this study proposes an alternation of drug delivery and "drug holiday" periods, the switch being determined here by the side effects of the cytotoxic drug on a healthy cell population.

Multiscale Modeling: Integration from the Single Cell to the Cell Population Level

Changes in the environmental metabolism, or mutations, or added drug pressure, change the differentiation phenotype in the well-known Waddington epigenetic landscape (1957) and were revisited by Sui Huang in a series of articles [82–84, 85•, 86]. In this metaphoric landscape, cells follow, from a pluripotent state, a differentiation journey that resembles the fate of a ball rolling in a succession of bifurcating valleys with apparent stochastic choice at each bifurcation. However, such bifurcations in differentiation are not random and depend on balances between dynamic determinants of gene regulatory networks that are not metaphoric, relying on identified biochemical mechanisms that can be modeled as bistable (or multistable) dynamical systems. In particular, epigenetic barriers in the landscape may be lowered, others raised, diverting cells from their normal differentiated states (the ends of the valleys, rolling down) and possibly trapping them in basins representing immature states, e.g., myeloblasts in the case of hematopoiesis. A way to represent such phenomena in mathematical models is to use, at the cell population level, PDEs structured in antagonistic variables, that are themselves at the single-cell level solutions of a dynamical (ODE) system on the parameters of which the tumor environment may act, as proposed in [87, 88], where an illustration was given on a model of differentiation of T cells between Th1 and Th2 cells, governed at the cell level by dynamic transcriptional regulation of transcription factors T-bet and GATA-3.

Conclusions and Possible Use of This Review

In this second paper, we have recapitulated, from a theoretical biology point of view, the main features of cancer cell populations that should be retained to understand drug resistance in order to design mathematical models to represent and analyze drug resistance, and predict its evolution. This interdisciplinary field of research also needs, in order to be properly explored in collaborative studies between biologists, oncologists, and mathematicians, a clear understanding of what different types of mathematical models can do and cannot do. This is naturally clear to mathematicians, and to avoid any misunderstanding, it should be made clear also to biologists before any common research effort is undertaken. This was the main purpose of this second part of our review, which we hope will serve as a preview for future interdisciplinary studies on stem cells and drug resistance in cancer in light of mathematical models.

Compliance with Ethical Standards

Conflict of Interest Aaron Goldman, Mohammad Kohandel, and Jean Clairambault declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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