Cell population heterogeneity and evolution towards drug resistance in cancer: Biological and mathematical assessment, theoretical treatment optimisation

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Abstract

BACKGROUND. Drug-induced drug resistance in cancer has been attributed to diverse biological mechanisms at the individual cell or cell population scale, relying on stochastically or epigenetically varying expression of phenotypes at the single cell level, and on the adaptability of tumours at the cell population level.

SCOPE OF REVIEW. We focus on intra-tumour heterogeneity, namely between-cell variability within cancer cell populations, to account for drug resistance. To shed light on such heterogeneity, we review evolutionary mechanisms that encompass the great evolution that has designed multicellular organisms, as well as smaller windows of evolution on the time scale of human disease. We also present mathematical models used to predict drug resistance in cancer and optimal control methods that can circumvent it in combined therapeutic strategies.

MAJOR CONCLUSIONS. Plasticity in cancer cells, i.e., partial reversal to a stem-like status in individual cells and resulting adaptability of cancer cell populations, may be viewed as backward evolution making cancer cell populations resistant to drug insult. This reversible plasticity is captured by mathematical models that incorporate between-cell heterogeneity through continuous phenotypic variables. Such models have the benefit of being compatible with optimal control methods for the design of optimised therapeutic protocols involving combinations of cytotoxic and cytostatic treatments with epigenetic drugs and immunotherapies.

GENERAL SIGNIFICANCE. Gathering knowledge from cancer and evolutionary biology with physiologically based mathematical models of cell population dynamics should provide oncologists with a rationale to design optimised therapeutic strategies to circumvent drug resistance, that still remains a major pitfall of cancer therapeutics.

1. Introduction

Cancer is a disease that has raised many questions and proposals to understand its evolution with time on the basis of biological observations and by using theoretical and mathematical tools. The obvious aim of such research is to circumvent cancer, *i.e.*, not necessarily to eradicate it, but often more realistically to contain it within admissible limits for long-term survival of patients with a good quality of life. Emergence of resistance to cytotoxic drugs - even to targeted therapies [67, 102] - in cancer cell populations is common in most cancers [228], and is one of the major pitfalls encountered in oncology (the other major pitfall being toxic side effects of drugs to healthy cell populations), as it induces tumour recurrence in spite of therapy and it limits life expectancy. Phenotype or genotype heterogeneity in tumours may account for such resistance [19, 39, 190].

This review is intended not only for biologists nor only for mathematicians, but for both, trying to bridge a gap on this specific topic between these two fields of science. We lead it from the point of view of applied mathematicians who have gathered as well as they can all available evolutionary, biological and biophysical knowledge relevant to model evolution of cancer cell populations, with the aim to optimise cancer treatments by taking into account the role of intra-tumour heterogeneity in the development of drug resistance. It is not its object to describe the *molecular* mechanisms of drug resistance in single cells, which have been investigated in detail in numerous articles [106, 107, 127] and books [184, 264]. Rather than on molecular mechanisms, we will focus on the micro-environmental conditions and evolutionary mechanisms by which biological variability with respect to phenotypes within a given cancer cell population, *i.e.*, intra-tumour heterogeneity, is implicated in the development of drug resistance, see Table 1. Genetic mutations, either preexisting in the cancer cell population exposed to the drug, or induced by micro-environmental perturbations due to the drug, have been proposed to account for intratumoral between-cell variability with respect to drug sensitivity [101, 100]. Hence, drug resistance has been attributed by some to somatic evolution whereby somatic mutations in isolated cells (the so-called "renegade cell" hypothesis) are followed by Darwinian selection of the fittest phenotypes [112, 113, 192]. A competing hypothesis stems from the framework of "tissue organisational field theory" (TOFT) [236, 237], proposing that the influence of a diseased surrounding stroma is the real determinant of carcinogenesis, whereas others try to reconcile the two viewpoints [20, 217].

The influence of the tumour micro-environment (for example vascularisation, lymphocyte and macrophage infiltrates, fibroblasts, adipocytes) is certainly not to

be neglected, as it contributes to intra-tumour heterogeneity [148]. In fact, exchanges with the micro-environment have been shown in numerous cases to be responsible for drug resistance in the tumour [118]. However, there are situations of drug resistance in *a priori* genetically homogeneous, but phenotypically diverse, cancer cell populations in which no stromal influence may be evidenced (*e.g.*, in a Petri dish) and genetic mutations are excluded since reversal to total drug sensitivity can be obtained after some time following drug washout [231]. Simultaneous evidence of the involvement of reversible epigenetic mechanisms (*i.e.*, mechanisms that control gene expression without changing the sequence of base pairs in the DNA) has been ascertained in such cases [231], which sets the origin of such heterogeneity at the level of epigenetic regulations, in other words it relates it to *cell plasticity* [76, 82, 181, 183, 203] with respect to phenotype. By cell plasticity, we mean here (at the individual cell level) the ability for a cell to partly reverse its differentiation fate (thus going back in the direction of a stem cell state), e.g., by mechanisms involving epigenetic enzymes, that may result at the cell population level in global adaptability to a changing environment in the organism.

Indeed, this may be achieved at the cancer cell population level by what looks like a risk-spreading strategy called in evolutionary theory *stochastic bet hedging* [18, 38, 62], *i.e.*, stochastic repartition of phenotypes in the cell population allowing it to face very diverse insults. It may obviously be obtained phylogenetically (*i.e.*, resulting from successive mutations) by the mechanism of evolutionary branching of genetically distinct subclones [113], but it may also be of non-genetic nature, due to random transcription (see in a microbiological context [78]) or to unequal distribution of proteins at mitosis [137]. Such stochastic phenomenon has also been proposed to account for heterogeneity with predictable drug resensitisation after transiently established resistance to cellular stress induced by anti-cancer agents [25].

In order to study heterogeneity in tumours, it is mandatory that biological observations and mathematical representations of phenomena are captured at the scale of cancer cell populations since at the single-cell scale between-cell heterogeneity is obviously meaningless. Conversely, from a practical point of view, it should be mentioned that attempts to characterise the expression of genes in cancer cells, both at the genetic and epigenetic levels, cannot rely on measurements performed on populations of thousands of cells, lest they yield average profiles that mask significant variations in the distribution of such expression [1, 7]. Indeed, even in genetically homogeneous cell populations, gene expression levels vary greatly between cells [6, 8, 190]. In order to apprehend such heterogeneity,

Table 1: Drug resistance in cancer: single-cell molecular and cell-population evolutionary viewpoints. "Cell-population representations" refers to representations that give rise to mathematical models structured in a continuously evolving trait, such as phenotype-structured partial differential equation models and agent-based models, see the last section of this review.

	Single-cell molecular representations	Cell-population representations
Pros	1. Close to cell biochemical reactions 2. Identifiable drug targets 3. Amenable to systems biology and (uneasy) whole body physiologically based pharmacokinetics-pharmacodynamics representations 4. Amenable to large network simulations 5. The actual levels of drug action	1. Amenable to cell physiology fate representation (proliferartion, apoptosis, differentiation) 2. Amenable to include phenotype evolvability 3. Simplicity of representation (lumped variables) 4. Amenable to mathematical analysis 5. The actual level of patient's disease observation
Cons	1. Many intricate cellular networks 2. Unable to integrate phenotype evolvability (Darwinian-like evolution of populations) 3. Qualitative cell physiological fates absent 4. Hardly amenable to mathematical analysis	1. Not close to cell biochemical reactions 2. Drug targets are not molecular, only functional (hence uneasiness to represent drug effects) 3. Hardly amenable to large network representations (hubs in large networks absent)

it is in our opinion mandatory to perform phenotype analyses at the single-cell level in the same cell population to reconstruct (by large sampling of individual cell data through, for instance, fluorescence-activated cell sorting) the *probability distribution of single-cell phenotypes across a cell population*.

A second reason to set the magnifying glass on cancer physiopathology at the cell-population scale is that, by taking into account heterogeneity at the cancer cell population level, it may be possible to explain why most anticancer drugs, even recently developed targeted therapies that try to hit intracellular pathways at supposed hubs, have generally and inexplicably led to so many treatment failures [67, 102, 228, 254] despite being seemingly efficient at the single-cell level. Although some targeted therapies such as in chronic myeloid leukaemia by inhibition of the BCR-Abl chimeric protein [74, 123] have been successfully used,

Table 2: Factors of between-cell functional heterogeneity in cancer cell populations. We distinguish between intra-tumoral factors that are (*A*) not directly concerning the tumour cell population itself but rather its local ecosystem, and (*B*) intrinsically linked to the tumour cell population itself.

A.	External to the cells constituting the cancer cell population
	1. Intra-tumour cellular interactions with stromal cells (non-cancer cells): vascularisation and its quality; cancer-associated fibroblasts (and adipocytes); infiltrated by lymphocytes and macrophages
	2. Local metabolic conditions: oxygen, glucose, acidity, growth factors
	3. Local biophysical conditions: pressure, temperature
B.	Characteristics of the cells constituting the cancer cell population (cell by cell)
	1. Cell status with respect to (de)differentiation: stem-like plasticity
	2. Cell status with respect to proliferation and apoptosis potentials
	3. Methylation status (stable/unstable) of DNA and histones
	4. Presence and quality of mitochondria; respiration/fermentation metabolism
	5. Presence and quality of functional gap junctions and maintained polarity

with usually few resistances found at the beginning of their introduction in therapy, most of them have eventually met this common pitfall of anticancer drug therapies [40, 114].

These observations prompt us to ask whether a cancer cell population within a tumour is so diverse that we should expect the presence of some resistant cells capable of giving rise to a progeny that is unbeatable by the drug at hand. In other words, is heterogeneity in cancer cell populations constitutive of the most incurable cases of the disease, and the reason for so many treatment failures? In general, heterogeneity in cell populations is not characteristic of a diseased state. Indeed, some between-cell heterogeneity is necessary for well-organised organs to perform their tasks [219]. It is only when such ordered heterogeneity is perturbed, in particular by loss of quality of intercellular communications that robustly structure a physiological tissue (see below *Biophysics and bioenergetics*), that it may become cancerous [220]. More precisely, it has been proposed that impaired gap junctions are often associated -through cause or consequence - with cancer in tissues [249, 250].

Here, we firstly review the different types of heterogeneity encountered in cancer cell populations and their known physical, chemical and micro-environmental

determinants, see Table 2. We then present evolutionary hypotheses that shed light on the phenotype plasticity of individual cancer cells and on the resulting phenotype adaptability due to phenotype heterogeneity of cancer cell populations. We discuss some general concepts regarding evolution and cancer including the Darwinian evolution of genomes *over the course of billions of years*, from unicellular organisms to mammals. This sets up a framework for discussing the first hypothesis - the so-called atavistic theory of cancer - which interprets *cancer as an archeoplasm rather than a neoplasm* [253]. We then show how the notion of an *epigenetic landscape*, initially described by Conrad Waddington [256] and recently revisited by Stuart Kauffman, Sui Huang and colleagues [36, 128, 129, 130, 131, 132, 133, 134, 135, 136, 203, 204], offers a complementary view that adds another necessary temporal component *on the time scale of a human life* to this elementary description of the conditions that determine the evolution towards drug resistance in cancer. Thus, following the metaphor of the epigenetic landscape and setting the problem of the evolution of cell populations within a given genome (one multicellular, in particular human, organism), we show how different and rich a perspective this notion of a moving epigenetic landscape adds to the notion of the Darwinian evolution of genomes. We also mention the importance, in this developmental process of the control of proliferation, of the immune response checking to eliminate accidentally occurring non-self elements, as a fail-safe complementary strategy when other controls have failed.

Finally, we briefly review some mathematical models that have been proposed to represent evolution in cancer cell populations and we discuss their possible use to set theoretical therapeutic optimisation in the framework of optimal control problems, focussing on continuous phenotypically structured models. The built-in targets for such theoretical therapeutic control in the models we advocate for drug delivery optimisation are not supposed to represent well-defined molecular effects of the drugs in use, but rather their functional effects, *i.e.*, related to cell death (cytotoxic drugs), or to proliferation in the sense of slowing down the cell division cycle without killing cells (cytostatic drugs given at low or medium doses), or even to phenotype plasticity reduction when such drugs are available. Waiting for combinations of chemotherapies and immunotherapies to be available and efficient in the clinic, we propose in the meanwhile that a rational combination of cytotoxics and cytostatics, relying on optimal control theory, may be optimised to propose therapeutic control strategies to confront the emergence of drug resistance in cancer and overcome its effects.

2. Heterogeneity in cancer, or the heterogeneous nature of heterogeneity

Although, as pleasantly stated by Sir David Smithers in *The Lancet* in 1962 [234],

> *"Cancer is no more a disease of cells than a traffic jam is a disease of cars. A lifetime of study of the internal-combustion engine would not help anyone to understand our traffic problems"*,

it is nevertheless possible to collect information about different characteristics of cells that may account for relevant biological variability in the cell population. Besides, in the biological case, such characteristics may be dependent on the cell population environment, which is not the case for the automobile metaphor.

2.1. Diversity of cell characteristics to describe variability in a cell population

Cell populations in tissues are characteristically diverse by many aspects, that can be physically measured such as location in space (when the cell population is endowed with a geometrical structure, as is the case of highly ordered organs such as the liver, or of tumour spheroids), pressure (*i.e.*, stress, when the medium is out of equilibrium, which is the case in growing tissues), pH, oxygen saturation, glucose concentration or concentration in metabolites that are relevant for biological phenomena under study. Other variables may also be used to characterise relevant biological variability at the level of a cell in a proliferating cell population, such as age (a lumped variable assumed to represent the sum of products of protein synthesis), size at division or at cell cycle phase transitions, and in any cell population, the expression of genes of interest, the activity of cellulular detoxification enzymes or membrane proteins such as ABC transporters [107], the determinants of energy metabolism (such as number and quality of mitochondria), to name but a few. Other characters may be visually assessable by pathologists, such as epithelial versus mesenchymal phenotypes for cells with the same local origin that may encompass transitions from one state to the other (EMT or MET) [208, 247].

This is physiologically true of tissues in healthy multicellular organisms as well as of cancer cell populations in growing tumours. Physiologically, all cells in a given healthy organism are endowed with the same genome, whereas solid tumours typically contain multiple genetic clones, and within these clonal populations there can exist phenotypic diversity across a range of functionalities and behaviours such as those mentioned above. Heterogeneity in cell populations in general is thus not pathological as such, provided that it is *ordered*. However, observed loss of communications between cells that ensure tissue coherence, *i.e.*, disordered heterogeneity, might constitute a main cause of cancer [220, 236, 237, 249, 250].

Table 3: Factors that determine the physico-chemical conditions for metabolic reactions in normal cells of evolved multicellular organisms and which are usually impaired in cancer. These factors have emerged during the course of evolution from the first Metazoa 1.0 (1000 million years ago, Mya) up to the Metazoa 2.0 (600 Mya).

A.	Energetic metabolism (1450 Mya)
	Within-cell normal respiration in mitochondria (tricarbolic acid cycle) or fermentation: dates to a time well-before multicellularity and likely came about through endosymbiosis leading to mitochondria-bearing cells
B.	Physics of multicellularity (850 to 545 Mya)
	1. Between–cell collagen glue constituting the extracellular matrix (ECM) 2. Adhesion to the ECM 3. Polarity in epithelial and other cells 4. Tight & gap (+++) junctions (collagen synthesis needs molecular oxygen; adhesion and dispatching of toxics between cells need junctions)
C.	The "multicellularity genetic toolkit" (1000 to 600 Mya)
	Genes contributing to physiological intracellular regulatory networks and to between-cell adhesion and communication physics (*B*), that emerged during the evolution from protometazoans (Metazoa 1.0) to evolved metazoans (Metazoa 2.0). Although precise descriptions of this 'toolkit' remain elusive – but would be very helpful to document the *atavistic theory of cancer* (see below) and orient therapeutics – one may without doubt mention among them *cMyc, p53, Hox, collagens, connexins*, often altered in cancer.

2.2. *Descriptive characters of a physical, chemical and micro-environmental nature*

We briefly mention local tissue characteristics related to physics, chemistry, or other micro-environmental determinants, known to influence phenotype diversity in cancer (see Table 3); as mentioned in the introduction, we do not detail the molecular mechanisms themselves that actually modify these phenotypes towards malignancy.

Biophysics and bioenergetics. Beyond profound modifications of tissue intensive variables (such as temperature, pressure, concentration in oxygen, glucose, cytokines [60], see below), between-cell communications (such as by variable polarisation of the external membrane [165], through microtubules [57] or gap junctions [93, 249, 250]) in cancer tissues have been reported, and even proposed to be among the causes of malignant transformation.

Poor bioenergetics of cancer cells might be responsible for such defects in information and energy transfer (that are indefectibly linked [188]) between cells in cancer tissues, and it has even been hypothesised that cancer may be considered as a disease of the mitochondrion. The absence of properly working mitochondria has consequences for information and energy transfer between cells since the ability to sustain high-performance intercellular communication that is needed in a well-organised tissue and involves expense of energy is then impaired. Other consequences of defective mitochondria include the so-called Warburg effect [213, 257] (see below) and the development of resistance to proapoptotic molecules, as the opening of mitochondrial pores, impaired in cancer, is a necessary step in the apoptotic cascade [159]. The cell mitonchondrial content itself is also a source of energetic heterogeneity [115].

One may also speculate that the scarcity and bad quality of intercellular information and energy transfer leads by natural selection to the *survival of the fittest tumours* in an organism, *i.e.*, of those that have stochastically developed a sufficiently varied fan of phenotypically diverse cells to allow them to survive, as tumours, the most different changes in their micro-environment defined by cellular stress (see below), resulting in stochastic bet hedging (see above).

Cellular stress. Modifications of the normal physical and chemical tissue conditions mentioned above, that imply metabolic conditions hostile to healthy cells, include hypoxia, acidity, local pressure increase, inflammation and insult by xenobiotics such as cytotoxic drugs. Tumour cells are more able to resist cellular stress than healthy cells. This is likely due to their plasticity and to bet hedging strategies

at the level of the cell population; furthermore, cellular stress may even cause cancer. For instance, increase in local pressure between cells has been experimentally evidenced to induce malignant transformation of the tissues [86]. It is also the fundamental hypothesis of tissue organisational field theory (TOFT, see above) that cancer is the result not so much of the progeny of a single renegade cell, but mainly of a diseased surrounding tissue engendering cellular stress [236, 237]. Another fact about cellular stress due to high doses of cytotoxic drugs is that it reduces phenotype heterogeneity in tumours by selecting highly resistant clones [67, 106].

Glycolytic metabolism. The Warburg effect is the switch, observed in cancer, from the normal oxidative phosphorylation phenotype, relying on the tricarboxylic acid (TCA, or Krebs) cycle occurring in the mitochondrion, that needs oxygen, to anaerobic glycolysis, that does not need it [186, 213, 257]. This switch allows cancer cells to survive in hypoxia; furthermore, anaerobic glycolysis produces lactic acid, which lowers extracellular pH and allows cancer cells to better survive in comparison to normal cells. It seems to be all the more active as hypoxia is localised in space [146] or intermittent [252] in tumours. An intensive switch to anaerobic glycolysis leads cancer cells to recruit glucose from alanin and glutamine in muscle proteins for their metabolism, resulting in cachexia in advanced cancers [93]; however in general, heterogeneity with respect to oxidative phosphorylation or anaerobic glycolysis exists in cancer cell populations [143]. An increase in anaerobic glycolysis has been shown to be associated with drug resistance, which has led to the proposal of targetting cellular metabolism as an innovative therapeutic strategy [262].

Epigenetic factors and cell plasticity at the gene expression level. Cell metabolism strongly conditions for the activity of epigenetic factors [83, 85, 84] that are responsible for plasticity in cancer cells, *i.e.*, reversibility to a pluripotent state characteristic of stem cells (within the course of differentiation) [44, 186], and connections between mutations of enzymes of the TCA cycle, such as IDH1/2 and epigenetic control of cell plasticity have been evidenced [235]. Furthermore, the so-called w factors [245], have been shown to reprogram metabolism [104]. These epigenetic factors (such as the DNA methyltransferase DNMT3A, which is often mutated in acute myeloid leukaemia [260]), control DNA methylation [145, 222], the intensity of which is related to gene expression silencing [224] including the silencing of tumour suppressor genes [121]. They may also contribute to the plasticity of tumour cells that can lead to phenotype heterogeneity [119, 227] within a cancer cell population, proposed to be a source of non-genetic insta-

bility [140], in particular with respect to the epithelial versus mesenchymal phenotypes [104, 246]. Pluripotent cancer cells, or so-called cancer stem cells, are also, due to such epigenetically controlled phenotypic plasticity, less sensitive than differentiated cancer cells to anticancer drugs [63, 72, 79, 258]. Note that the *molecular* mechanisms of epigenetic dynamics, which are beyond the scope of the present review, have been partly unravelled in [31].

Cellular tumour micro-environment and the cancer niche. The tumour micro-environment is the local ecosystem in which tumour cells thrive [148], thanks to supply by other cells in oxygen, nutrients, inflammatory cytokines or metabolic factors. The vasculature is of importance to maintain this micro-environment, although cancer cells are endowed with the ability to survive in hypoxia, thanks to the Warburg effect mentioned above. Also of importance are infiltrated lymphocytes and macrophages, participating in local inflammation, fibroblasts that may be transformed into cancer-associated fibroblasts [32, 49, 147, 176] or in specific tumours (breast, prostate) adipocytes transformed into cancer-associated adipocytes [68, 160]. It has been proposed to make use of physical means specifically targetting the micro-environment with the aim to revert cancer tissues to normal [138]. The cancer niche, on the other hand, consists of non-cancer cells living in symbiosis with tumour stem cells [13]. It is an emergent micro-environment in tumorogenesis with similarities to the normal micro-environment of stem cells, whose existence is enforced by the proximity of cancer cells themselves, and that is conversely conducive to cancer cell survival and proliferation. It is not a part of a cancer cell population, hence, strictly speaking, it does not participate in its cell heterogeneity. However, the alteration of the niche through the actions of both cancer cells and non-cancer cells can modify the natural selection pressures that act on these resident populations. According to niche construction theory [193], this type of dynamics can have an evolutionary effect when the modified selection pressures persist in a localised manner over multiple cell generations. The cancer niche may favour those stem cells that have evolved towards malignancy [42, 251].

3. Evolutionary mechanisms that contribute to heterogeneity in cancer

Cancer is an evolutionary disease, in the sense that not only can some of its causes and determinants be found in the history of the evolution of species, but also because cancer cell populations most often evolve their phenotypes when they grow in size and malignancy, acquiring de novo properties that render them able to escape all controls and ultimately invade their host organism. The idea of cancer

as an evolutionary disease is certainly not new, and it has been set under focus by many in the last decades [5, 113, 187, 192, 218, 238, 241]. However, theoretical views on evolution in cancer cell populations that go beyond the description of evolving genetically distinct cell clones are not so broadcast. Yet they may help to explain the naturally heterogeneous nature of cancer cell populations and their ability to develop drug resistance.

Letting the readers interested in the general theory of evolution to deepen their views by consulting the works of Charles Darwin [58], Steven Jay Gould [108, 109, 110], John Maynard Smith [179, 180] and others, we begin by general considerations on evolution and cancer, and then review in more detail three aspects of evolutionary theory that shed light on our subject: a) the so-called *atavistic theory of cancer* [60], b) the epigenetic landscape, starting from Waddington until his recent epigons, around Sui Huang, and c) hints about the evolution of the immune system and its possible use in the clinic, "targetting cancer's weaknesses rather than its strengths" [168].

3.1. Introducing an evolutionary perspective in cancer biology

"Nothing in biology makes sense except in the light of evolution" (Theodosius Dobzhansky) [69], and this is particularly true in developmental biology and in cancer, that is firstly an evolutionary disease, more precisely *a disease of evolutionary multicellular organisation* because it may be considered as a backward step in the course of evolution towards organised multicellularity, according to the atavistic theory of cancer [60] (see below).

Cancer thus represents evolution, anatomically localised in the organism (initially in a given organ, possibly extended to other tissues by remote metastases), of *genetically new species* developing, likely with branching [113] by successions of mutations, their diversity at the expense of the host organism. We contend that the disappearance of successive physiological control mechanisms described below puts cancer cell populations in the state of a very primitive multicellular organisation.

Evolution from unicellular to well-organised multicellular organisms (such as mammals) has involved in the course of billions of years layers of growing complexity which support their proper function. These include: 1) the simple regulation between proliferation and apoptosis at the single cell level to allow a population of initially identical cells to expand and survive in changing microenvironmental conditions; 2) the development of specialisation in cells (already evidenced at elementary levels of evolution towards multicellularity, for example,

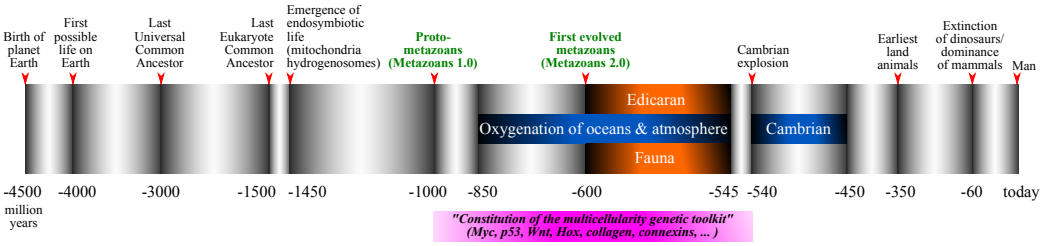

Figure 3.1: *Tentative approximate reconstruction of the evolution of life on planet Earth, from [22], [60], [103], [125], [152, 153], [157], [168], [171], [177], [194], [215, 216], [223], [226], [233], [248], [249, 250], and others. Time is in millions of years (My). We have only set on this rough timeline widely known biological and geological episodes such as in the late Precambrian the Ediacaran [156] fauna, first metazoa 2.0 that needed atmospheric or oceanic oxygen and the so-called Cambrian explosion [109]. Although oxygenation of the oceans and atmosphere began before -850 My, we have mentioned here only the period during which it became significant enough to allow for the progressive constitution of evolved metazoans [125] (see also [195]). In the perspective of this "multicellularity genetic toolkit" well established by -600 My, nothing fundamentally new for genes altered in cancer is hypothesised to occur after the so-called Cambrian explosion; only the body plans of animals may have then encompassed considerable enrichment, see [90] for a biophysical hypothesis on this subject. See also [225] for a hypothesis on the transition between the extinction of the Ediacaran fauna and the Cambrian explosion.*

in colonies of *Dictyostelium discoideum* [2], or bacterial films [117]) to yield differentiated cell states with different functions; 3) the development of higher levels of control such as transcription factors to organise and regulate such differentiation; 4) the control of transcription factors by epigenetic (genes and resulting) enzymes in a coherent way to achieve harmonious functioning between the differentiated cell populations; and 5) the immune system to eliminate possible errors in proliferation when it becomes uncontrolled. Indeed, in the evolution of genomes from unicellular to sophisticated multicellular organisms, proliferation – which is the fundamental biological mechanism through which life exists as such – has been physiologically more and more strictly controlled by transcription factors and epigenetic mechanisms that ensure the cohesion of such multicellular constructions. Note, however, that, sophiscated as they are, these layers of control do not result from the design of a harmoniously conceived plan, but rather, as pointed out by François Jacob, from 'tinkering' [142], and that, as in all tinkerings, local failures are always possible. Local escape from these controls during cancer, resulting in uncontrolled proliferation in anatomically iso-

lated parts of the organism, has been hypothesised to be a localised return to the ancestral state of unicellular organisms or, rather, to very elementary forms of multicellularity [59, 139, 168, 242, 243, 253, 254] ("Metazoa 1.0", see below about the atavistic theory of cancer). This has been individualised in cancer as an intermediate state of coarse cooperation between grossly differentiated cells in a tumour [144, 178, 207, 244], that organises itself to survive as an independent entity.

The Darwinian evolution that leads to the constitution of genetically well defined genomes, adapted to their (macro-) environment (*i.e.*, in this occurrence, on our planet Earth) should be clearly distinguished from evolution – in a general sense – in the phenotype of cells *within a given genome* in the corresponding multicellular organism (one of us). This physiological differentiation and maturation of cells within one genome occurs over the timeframe of a human life, not of billions of years, and can be metaphorically represented by Waddington's epigenetic landscape [256].

Following this metaphor, the epigenetic landscape is initially (in the embryo) the same for every tissue to be. However, as the organism develops, so too do the epigenetic landscapes for each tissue through a process of local adaptation. In particular, an epigenetic landscape is *locally* (in an organism) modified when a mutation occurs in a given genome in an isolated part of the organism; conversely when it is energetically too costly for the local landscape to maintain epigenetic barriers that ensure its cohesion under repeated attacks (of cellular stress, in particular by anticancer drugs), natural selection in the population of dividing cells may act on viable epimutations (at the molecular level, usually by methylation of the DNA), or even mutations, yielding locally (in the landscape) new epigenetic barriers that are adapted to the stress and solidly established. This point of view, that focuses on irreversible genetic changes as a response to cellular stress, has been popularised by Henry Heng and colleagues, using the term of "genome chaos" [122, 126, 169]. However, even in this perspective, drug-induced drug resistance, in as much as it may have been shown to be due to mutations, may have been preceded by transient epigenetic modifications [155], while in other cases it may rely only on such epimutations, without established genetic mutations. In both cases, such perspective opens up paths for the investigation of innovative cancer therapies relying on modifying epigenetic regulations either directly or indirectly by acting on the cell metabolism on which they depend. In fact, genetic and non-genetic instability seem to be not independent of one another [232]. Even only epigenetic modifications, i.e., without mutations, can be responsible for firmly established drug resistance [37]. Can epimutations furthermore pro-

voke subsequent mutations on the same genes? It has been proposed that mutations occur before the onset of epimutations [206], but it has also been reported that epigenetic silencing by methylation makes single nucleotide C to T mutations on the DNMT3A locus highly probable, entraining in turn more epigenetic alterations [261]. This example at least may give some consistency to the hypothesis of mutations being possible consequences of epimutations, and, metaphorically, cellular stress being a possible cause of firstly transient and subsequently irreversible modifications of the landscape.

An interesting observation is that when there is actual development of genetically distinct resistant subclones, their number does not grow indefinitely; in fact, only a relatively small number of them survive [67]. This has been explored by using mathematical modelling approaches [240, 255]. However, the mechanism of such evolution towards a relatively scarce number of resistant subclones remains unknown. One might speculate that mutations yielding defects in DNA repair enzymes can produce different mutations in different parts of the DNA, some of which only proving to be viable in the tumour mass, due to natural selection. Biological evidence is to our best knowledge still lacking to support such a hypothesis.

3.2. Evolution from unicellular organisms and the atavistic theory of cancer

Noting that some cohesion, even cooperativity, between cancer cells always exists in tumours [144, 178, 207] does not shed much light on tumorigenesis. Therefore, to understand the role of cohesion in tumorigenesis, we shall review hypotheses about the emergence of such cooperativity.

Self-organisation has been evidenced in primitive forms of interaction between agents supposed to represent the "Game of Life" invented by John Conway in 1970 [12]. The Game of Life is a cellular automaton (*i.e.*, a program) constituted of agents that can show multiple global patterns according to the rules that define it. Of note, the structures that emerge during the Game of Life are only dynamic and represent far from equilibrium dynamics. These can present what physicists call *criticality*, and inevitably evolve towards more complex forms of organisation. This is a phenomenon similar to abrupt phase transitions that are well known in physics, and are reversible but potentially with a high energetic cost for such reversion. In the theory of evolution that we are interested in here, such phenomena have been depicted by John Maynard Smith and Eörs Szathmáry under the term of major transitions in evolution (MTE) [179, 180, 195].

It has been proposed that such a transition took place when amorphic agglomerations of single cells first evolved into primitive, but robust, forms of multicel-

larity ("proto-metazoans, or Metazoa 1.0" according to Paul Davies and Charles Lineweaver [59, 226]) that could spontaneously show patterns favouring communication between cells, possibly due to the enrichment of the atmosphere in oxygen and collagen formation, that needs molecular oxygen [226].

Multicellular life is supposed to have remained in this primitive state until more organised forms of multicellularity ("Metazoa 2.0") emerged, about 600 million years ago, see Fig. 3.1, with extended differentiation processes and their epigenetic sophisticated controls, eventually yielding modern animals by silencing many of the genes on which the existence of Metazoa 1.0 relied. This second transition, to Metazoa 2.0, shows the same criticality as the first one, and is also reversible. The idea that oncogenesis is a tissue phenomenon presenting many characters of a phase transition is developed in detail from a physicist's point of view in [60]. In the atavistic theory of cancer, oncogenesis is likened to a local reversal of this phenomenon [60]. Specifically, cancer is seen as a result of a backward evolution from a tissue normally constituting sophisticated Metazoa 2.0 to the more robust Metazoa 1.0 (their atavistic ancestors [59]), which qualifies cancer to be *an archeoplasm rather than a neoplasm* (Mark Vincent [253]). By "archeoplasm" is meant a reversion, or backward evolution, to an archaic, early multicellular, form of life. This viewpoint has recently been experimentally assessed in mice [45].

Each cell endowed with a given healthy human genome bears all programs that can lead to the terminally differentiated cell states in the adult organism. According to the atavistic theory, we bear in particular in our genome all repressed elements of genetic material ("unused attractors", according to Sui Huang, in the epigenetic landscape created in each one of us constituted as a coherent multicellular organism, see below) that, in the absence of sufficient control, can be de-repressed and set to work, resulting in cancer as a de novo Metazoon 1.0 inside a Metazoon 2.0 organism. The risk of its occurrence is thus the price we must pay for the constitution of sophisticated coherent multicellular organisms [59, 70, 71], which are dynamic constructions always far from equilibrium [210].

The internal coherence between cells and organs of a healthy multicellular organism is hence non-genetic (possibly epigenetic or of a totally different nature [205]) and it relies on dynamic programs that may have a limited – in time – role to play (in embryogenesis and morphogenesis [165]) or on the contrary for some of them, are conserved in active form in stem cells in the adult and can produce organisation at the tissue level (TOFT [236, 237]). Such coherence in the differentiation of cells is initiated from totipotent cells in the first stages of embryogenesis, letting subsist in the adult only a limited number of pluripotent stem cell types,

corresponding to the great functions of the organism (haematopoietic stem cells, intestinal stem cells, cardiac stem cells, and many others). These dynamic programmes have epigenetic enzymes as their effectors at the gene expression level, and mutations in their genes, such as TET2 or DNMT3A, are frequent in early oncogenesis [64, 211, 212, 235, 261].

Note that in the framework of this evolutionary schedule that may explain cancer and its inevitable increase in tissue heterogeneity when regression to Metazoa 1.0 occurs, open questions remain: apart from Darwinian selection that has eliminated non-viable multicellular organisms in the course of evolution to Metazoa 2.0, what mechanisms keep together so phenotypically different cells in a viable Metazoon 2.0 organism (about 200 different cell types in a human body[1]). On what informational grounds is such coherence based? Is it synchronisation between cells? Are circadian clocks and the central circadian pacemaker (known to be disrupted in cancer, all the more so as the cancer is more advanced, such enhanced disruption resulting both in poor prognosis and poor response to anticancer drugs [164, 221], which has been also evidenced experimentally in mice [88, 89]) actual effectors and organisers or plain witnesses of such coherent organisation? Or is such cohesion due to the transfer of information on principles of classic thermodynamics [93] or of quantum mechanics [205]? In the metaphoric Waddington's landscape (see below), quasi-potential barriers establish tissue differentiation with normal cooperation between cells within coherent tissues constituted as organs, and between organs that build together an organism; however this says nothing about the mechanisms of the global cohesion of the scenery.

What leads such cohesion, certainly necessary for health, but still not explained, astray in cancer? According to the fundamental theoretical work of August Weismann (1834–1914), the only mission of the *soma* of sexually reproducing animals is to serve, preserve and transmit the *germ line* (or germ plasm, *i.e.*, the genome as contained in germinal cells). This needs a high degree of coherence in the soma of a multicellular organism for it to be able to faithfully fulfill such "dirty work" [105] without errors. Tumours are in this perspective local cheater tissues (selfish *cell populations*) that unfaithfully leave this cooperative program to proliferate in an organised form for themselves. To this end, they unmask genes that have normally been silenced in individual cells since the transition to Metazoa 2.0, and this results in escaping normal antiproliferative and differentiating messages that are necessary to maintain multicellular coherence.

[1] as reported *e.g.*, in https://www.unifr.ch/biology/assets/files/albrecht/lectures/chapter23.pdf

Such regression also allows cancer cells to use primitive defence mechanisms, that have normally been silenced in Metazoa 2.0, but were atavistically designed for Metazoa 1.0 cells to survive in hostile conditions, such as hypoxia, starvation, or ionising radiations [59]. These mechanisms lead to stochastically organised (with respect to phenotype) tumour cell populations (because of the poor quality of intercellular communications), although this may be efficient due to bet hedging (see above). Such an explosion of stochastic functional heterogeneity includes tolerance to xenobiotics, *i.e.*, drug resistance.

With this evolutionary perspective in mind, cancer is thus a disease, certainly not only of a single "renegade cell" (nor of a whole organism), but rather of a cell population localised in an organism. Tumours are not collections of identical cells that plainly proliferate. Not only is the distribution of their phenotypes nonuniform among cells in a given tumour, but also phenotype heterogeneity in these cell populations is meaningful, as they are at least roughly organised. Indeed, cooperation between cancer cells in a tumour exists [53], and it likely relies, as mentioned above, on reversion to an atavistic, primitive and robust form of multicellularity. As such forms of multicellularity do not need (and escape) sophisticated controls that physiologically ensure coherent organisation at the level of the organism, much room is left for increased heterogeneity in cancer cell populations. In fact, such heterogeneity may have been selected for in early evolutionary life as a risk-spreading strategy in fluctuating environments [18, 38]. This may be true of all forms of between-cell heterogeneity in cancer tissues mentioned earlier, and in particular of cell plasticity that may open the way for some cells in a population to develop a drug resistance phenotype.

3.3. Evolution in a given genome: Waddington's epigenetic landscape revisited

The epigenetic landscape imagined by Conrad Waddington as illustrated in his book of 1957 "The strategies of the genes" [256], see Fig. 3.2, was originally a simple graphic metaphor of cell differentiation fate. It is intended to represent *at the cell population level* cells on their way to differentiation from the stem-cell state as balls rolling down a scenery of bifurcating valleys, the last subdivisions of which end in fully differentiated states. It has been revisited by Sui Huang and colleagues in a series of articles [128, 129, 130, 132, 131, 133, 134, 135, 136, 203, 204], which link the bifurcations occurring between the valleys of the epigenetic landscape to bistable – or multistable [81] – switches in ordinary differential equations of systems biology representing the expression of antagonistic genes, such as PU.1 and GATA1 for the choice between myeloid and erythroid fates in the haematopoietic differentiation tree [46, 111, 136].

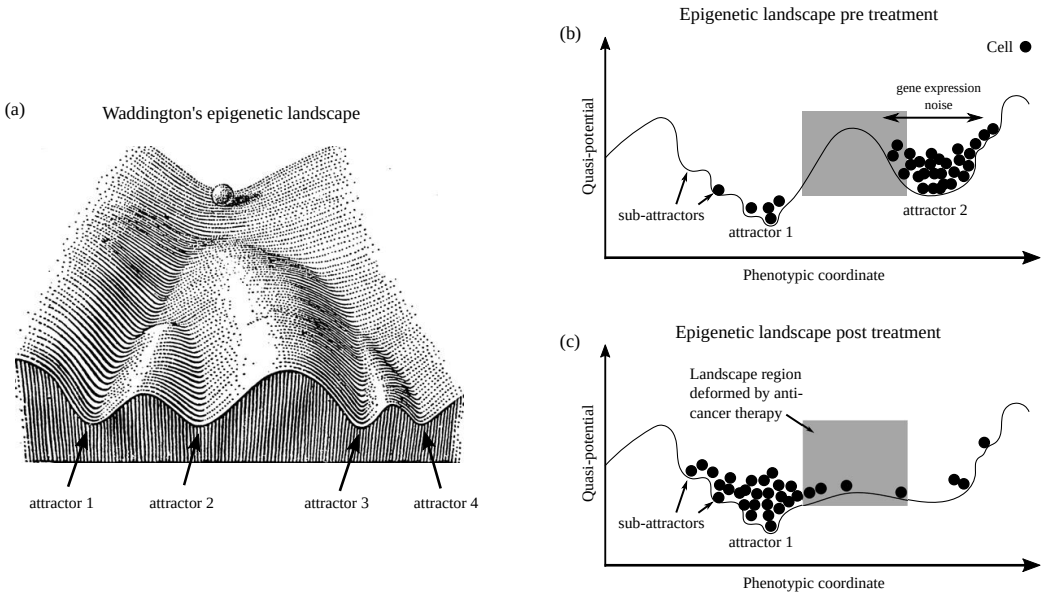

Figure 3.2: *The Waddington epigenetic landscape (*a*), and one of its possible interpretations as a soft, plasticine-like sculpture, moving under environmental - in particular drug - pressure: the delivery of anticancer drugs may lower normally constituted epigenetic barriers (*b*), allowing cells to trespass them and fill "unused attractors" (*c*), a scenario proposed by Sui Huang, see, e.g. [133].*

Sui Huang proposes, reversing the quote of Theodosius Dobzhansky [69], that "nothing in evolution makes sense except in the light of *systems* biology" [132]. Such representation and its mathematical formalisation are designed on the principle of a quasi-potential mimicking gravity, at its highest in stem cells and at its lowest in fully differentiated cells [263]. Note that in the framework of dynamical systems, the classic Waddington epigenetic landscape is compatible with the concept of pitchfork bifurcations (see, for *e.g.*, [73] or [200]) where two valleys are created from one. However, it has been suggested that cell differentiation should instead suppress a valley; rather than leaving the choice berween two valleys at bifurcations corresponds better to a saddle-node and not to a pitchfork bifurcation [87].

Let us stress that, unlike a genetic landscape describing all genomes in a population of individuals to highlight their relative fitness, the epigenetic landscape is attached to one given genome [133]. Metaphorically describing differentiation of somatic cells in a given tissue, it should be thought of as a moving, plasticine-like

landscape. Its motion depends on changes in gene expression, due to reversible epigenetic changes, that are influenced by the tissue metabolic environment (and cytokines or other determinant molecules) and to possible irreversible local mutations.

When the quasi-potential hills that separate valleys are of a genetic nature, they are hard-wired and impossible to move (*i.e.*, the plasticine is dry and as rigid as rock). However quasi-potential hills of an epigenetic nature can be lowered or heightened through, for instance, the silencing of gene expression. In the same way, plasticity with possible reversal to less differentiated cell states (*e.g.*, experimentally, at the single-cell level, making use of Yamanaka factors [245], or conceptually, at the cell population level, by cellular stress-induced epimutations and selection [48]) can be represented by changes in the quasi-potential of differentiation that alter the direction of its gradient (*i.e.*, reverting the flux of differentiation). Note that from a modelling point of view, according to the tissue and to the epigenetic control under study, one can design by equations relevant local parts of such landscapes ("Draw me a landscape" [191]) to help predict cell population fate under the influence of factors modifying the quasi-potential of differentiation.

The basic sculptors of such moving landscapes are (not forgetting the Yamanaka factors used to produce experimentally induced pluripotent stem (iPS) cells [245]) at the gene expression level epigenetic enzymes, already mentioned earlier about metabolism. Epigenetic events result in single cells at the gene expression level from methylation or demethylation of histones or bases, and they are under the dependence of these enzymes, among which are known in particular demethylases (HDMs), acetyltransferases (HATs) and deacetylases (HDACs) for histones, and DNA methyltransferases (DNMTs) or DNA demethylases (such as TET2 [158]) for the DNA (reviewed, *e.g.*, in [121]). At the cell population level, these enzymes are not necessarily homogeneously distributed in cells and can alter gene expression. Therefore, extended heterogeneity in the fitness levels of cells is possible, and this, in a given environment (such as cellular stress due to drug insult), can determine the evolutionary trajectory of a whole cell population. Nevertheless, such evolution is reversible since all epigenetic events, based on methylation or acetylation of aminoacids on histones or gene methylation on the DNA, are reversible.

With this perspective, phenotype plasticity in cells may be speculated to be a capacity to quickly unmask normally silenced genes, which can be related to *e.g.*, heavy loads of histone aminoacid demethylases and DNA methyltransferases (such as KDM5A or DNMT3A) in individual cells. Indeed, if such special cells exist in a cell population (and there is no reason, on the basis of a stochastic, un-

controlled, distribution of protein load between cells – see above about bet hedging [18, 38] – that it should not be the case in cancer), they should be candidates, rare as they may be, for the most highly plastic cells that can quickly adapt by unmasking atavistic resistance genes to face a life-threatening event. Such heterogeneity may help to explain reversible drug resistance phenomena as observed in [231], and reviewed in [76].

Indeed, when cancer cell populations are confronted by life-threatening events, such as high doses of cytotoxic drugs (as in [231]), it is highly likely that, due to their plasticity, which is mainly of an epigenetic nature, some of them (a tiny proportion is enough) develop a drug-resistant phenotype that can later expand and drive repopulation; this has also been recently hypothesised in [120] about resistance to EGFR antagonists. It has also been proposed that even when the drug concentration is only locally high, this may yield resistant cells that subsequently diffuse to zones in the tumour where less hostile conditions (in drug concentration) prevail, and eventually contribute to the development of global resistance in the tumour [94].

3.4. Evolution of the adaptive immune response in multicellular organisms

Heterogeneity – and reversibility – of the distribution of surface antigens in a cancer cell population, a feature that may account for resistance of cancer cell populations to monoclonal antibodies, has been known for some time [77, 80]. Although evolution of the immune system is not, strictly speaking, a feature of heterogeneity in cancer cell populations, it should be mentioned *en passant* in the perspective of theoretical treatments of cancer that take an evolutionary perspective into account.

In the framework of Darwinian evolution in multicellular organisms, *adaptive* immunity is among the most achieved and sophisticated means designed to fight cancer progression, even though native immunity is known to exist in primitive forms of multicellularity, such as sponges [239] . The most effective countermeasures of cancer cells are either to mask their surface antigens (by internalising them) or to increase the heterogeneity of their distribution in the tumour, in another bet hedging strategy against their attack by immunocompetent cells. The adaptive immune response is also a weapon that tumour cell populations cannot develop in an analogous way for themselves.

With this evolutionary perspective, it has been proposed that cancer treatments should not so much focus on fighting proliferation of cancer cells – as their return to an atavistic state of evolution renders them very powerful to resist cytotoxic

insults (as stated in [203]) by, for instance, maintaining drug-tolerant subpopulations as long as they need it – but on targeting cancer weaknesses (immunodeficiency) rather than its strengths (proliferation unabashed) [168]. Along this line, it was proposed, firstly and long ago by William Coley, who is considered as the father of immunotherapy (reviewed, *e.g.*, in [198]), to use pathogenic agents against cancer, and later, as a practical way to increase the differences in therapy sensitivity between healthy and cancer cells. For the latter proposal, the aim is to expose healthy cells and the naturally immuno-deficient cancer cells to an active pathogenic agent which, due to vaccination against the same agents, renders only healthy cells immunocompetent to defend themselves from the viruses [150, 168]. Oncolytic viruses, that are active against cancer cells, but harmless to healthy cells, are also an active subject of research nowadays (see, *e.g.*, [259]). Another treatment proposal is to first administer a combination of cytostatic and cytotoxic drugs to reduce the tumour burden, and then to deliver therapies which boost the immune system, with the hope that the immune response will be less overwhelmed by a heavy and heterogeneous mass of tumour cells [10, 230].

4. Mathematical models to predict treatment outcomes and drug resistance

4.1. What sort of heterogeneity, and what type of mathematical model?

Compared with the quantity of biological observations as well as of evolutionary observations and hypotheses on the involvement of heterogenity in drug resistance that have been presented in the previous sections, studies of mathematical nature are thus far scarce. However, in particular in the last decade, mathematical models coming from ecology, namely models of adaptive dynamics [65, 66], have been developed to take account of drug resistance in cancer cell populations by proposing cell population models structured by traits to describe relevant heterogeneity in those cell populations. Furthermore, optimal control methods [26] are also being proposed to circumvent drug resistance by combined therapies.

The choice of which between-cell heterogeneity to incorporate into a mathematical model of carcinogenesis will be dependent on the question of interest, the biological observations of the system, available data, and whether it is likely to be influencing, to a large degree, the observed dynamics. When studying evolutionary dynamics of cancer, it is also important to consider whether this heterogeneity is itself evolving, and if this evolution is occurring on a relevant timescale. For instance, the development of reversible drug tolerance (through epigenetic mechanisms) in cancer cell populations can occur over the timeframe of days [231],

whereas 20 years can elapse between carcinogen exposure and the clinical detection of a solid tumour [170].

What type of between-cell heterogeneity should be represented? Structure variables describing biological variability in physiologically based models may include relevant phenotypes such as size, age in the division cycle since last division, stemness markers, epithelial vs. mesenchymal visual shape, or any cell character relevant to the question at stake, see an illustration on some of the deterministic models that have been proposed on Fig. 4.1. The question of whether or not to include spatial dependence in the model to choose is also dependent on the conditions in which the biological phenomenon of interest is observed. Cells in the centre of a tumour, for instance, will have a very different set of selective pressures from those at its periphery, and in this case it makes sense to include space when some geometry of the tumour is known [141] – which is far from being the general case. When modelling cells in a Petri dish, on the other hand, it may be sufficient to assume that the cells are well-mixed and thus equally exposed to their environment.

4.2. What mathematical models should be used? Deterministic or stochastic?

Clearly, as we focus on *evolution* towards drug resistance, whatever the type of heterogeneity considered, it should not be static, but amenable to changes with time (evolution in the general sense). This point of view excludes purely static models of heterogeneity, and it favours models based on differential equations (ODEs), integro-differential equations (IDEs), partial differential equations (PDEs) or stochastic differential equations (SDEs). Stochastic agent-based models (ABMs) may also be used, endowed with evolution rules that make them useful tools to simulate tumour growth or other phenomena, however are much harder to analyse than differential models[2]. Among deterministic models, a fundamental difference exists between ODE compartmental models, in which heterogeneity is represented by the number of compartments, inside each one of which there is no variability (*i.e.*, total homogeneity is present), and PDE models, in which variability is present everywhere and is represented by a continuous (possibly multidimensional) structure variable, that may be spatial or phenotypic, or both (see Fig. 4.1).

[2]in particular, because no deterministic predictability based on the Cauchy-Lipschitz (also known as Picard-Lindelöf) theorem, *i.e.*, nothing like existence and unicity of the trajectory solution to a differential equation with given initial conditions, exists for them

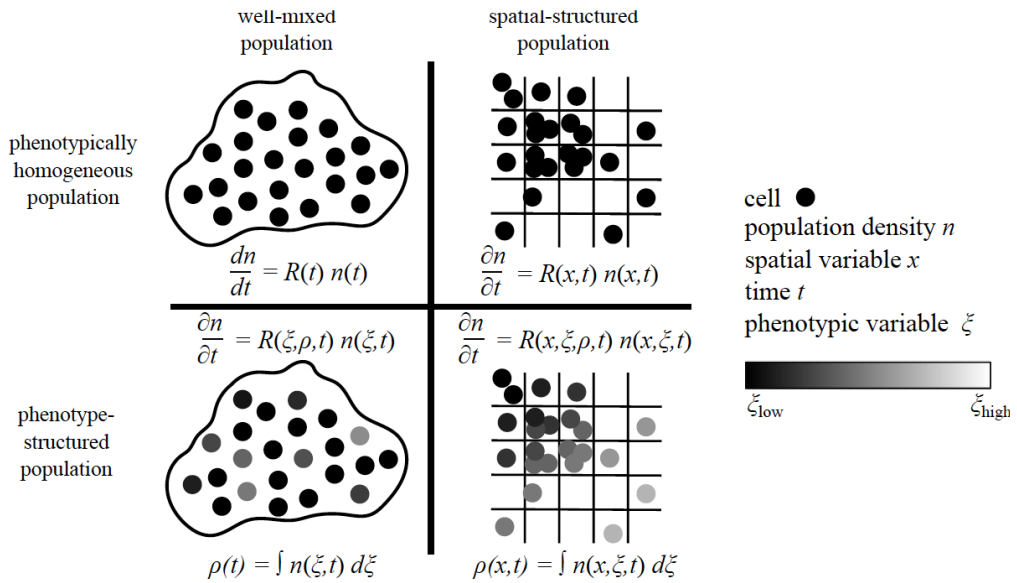

Figure 4.1: *Deterministic mathematical models adapted to describe cell population growth. Ordinary Differential Equation (ODE) Models are not* per se *amenable to describe evolution of heterogeneity in cell populations, except by juxtaposing them in compartmental models, with exchanges between them, thus representing evolution only in discrete steps (see below Fig. 4.2). Spatially or phenotypically (or both, mixed) structured models, on the other hand, can represent evolution in a more realistic way, taking into account continuous variations of the biologically relevant structure variables, i.e., 2D or 3D space x and / or continuously evolving phenotype ξ due, e.g., to dynamic methylation of histones or of the DNA in each single cell. Mixed discrete and continuous models are also available, as for instance continuous age-structured models (McKendrick-like transport equations, see below) of the cell cycle with discrete phases* G1, S, G2, M *and exchanges between them at cell cycle phase checkpoints [27, 28, 29, 30, 201].*

In general, deterministic models are useful for modelling the expected behaviour of population-level phenomena, such as the emergence of resistance or the formation of large-scale spatial patterns. Studying the asymptotic (*i.e.*, long-time) behaviour of a system is important, and for this reason deterministic models have the advantage of being readily amenable to asymptotic analysis by using many available methods. ABMs, on the other hand, are typically better at modelling a small number of (cell, in our case) entities where stochastic effects have a greater importance. They also offer a more natural description of a system, are more flexible than deterministic models while still capturing emergent evolution-

ary phenomena. Unlike deterministic models, ABMs can capture extinction and the occurrence of unusual events; however, they are generally more computationally expensive, which imposes limitations on the size of the population modelled. A study comparing ABMs and PDE models is [41]. Hybrid mathematical models (see below), that may mix – according to the scale and the environment considered – ODEs, PDEs and ABMs or probabilistic models, are another option for modelling cancer progression and response to treatments. These types of models are often useful when tissue-level dynamics (represented by PDEs or ABMs) are influencing dynamics at a cellular scale (represented by ODEs). This is the case when, for instance, intra-tumour heterogeneity, extrinsic to cells, such as local nutrient and metabolite concentrations, pressure, spatial position, etc., are influencing cell behaviours.

Beyond the question of the type of variables used to structure the model and the type of model (deterministic or stochastic) lies the question of *how* the dynamics of the physiopathological system itself must be represented in the equations, according to how close to the molecules one intends to describe it. In more detail, what are the reciprocal influences between different types of cell populations: are they due to direct competition (as, *e.g.*, in the case of tumour cells and NK lymphocytes) or to intercellular signalling molecules, how do they proliferate and die, and how are these dynamics influenced by environmental conditions? The closer to the molecular level, the more difficult it is to take into account all interacting agents by identifying parameters of the dynamics, and this can hardly be done beyond the single-cell level. As we advocate the cell-population level as the only really relevant one to mathematically describe between-cell variability and its evolution, we are led to favour a *functional* representation rather than a *molecular* one to describe the dynamics [27]. For instance, rather than representing the detailed chain of pharmacokinetic reactions from the infusion of a cytotoxic molecule in the general circulation until actual physical changes in cells (*e.g.*, creation of double-stranded breaks in the DNA), one may represent in a mathematical model its effects by an increase in the death rate at the cell-population level.

It is nevertheless possible, in cases where the single-cell molecular level and the cell-population level must both be considered, to integrate ODEs at the single-cell level into physiologically structured PDEs at the cell-population level (*e.g.*, T-bet and GATA-3 in helper T-cells, variables at the former becoming structure variables describing between-cell heterogeneity at the latter, examples cited in [91, 92]). However, such upscaling should be focussed on a specific phenomenon of interest, in particular cell fate balance due to antagonism of expression between two competing genes. Another way to proceed is to use hybrid ODE-PDE-ABM

models, with ODEs at the single-cell level, PDEs for diffusion of molecules in the interstitium, and an ABM to represent proliferation at the cell-population level, as advocated, *e.g.*, in [9], nevertheless yielding quite complex models to analyse and simulate.

We will illustrate in the following sections these ideas by discussing some of the models published in the literature.

4.3. Some mathematical models to represent heterogeneity in cancer

Probabilistic and hybrid probabilistic-deterministic models. Probabilistic models stated in terms of branching processes, that recapitulate a famous drawing by Charles Darwin in his notebook of 1837 mentioned in [113], are commonly used to study cancer evolution (that leads to stochastic bet hedging in tumours) in particular by the accumulation of passenger and driver mutations during tumour growth [34, 35]. Such models are based on the assumption that cell behaviour is fully determined by cell-intrinsic properties and, as such, a cell will behave in the same way alone as it would as part of a population. Therefore, while branching processes can describe the evolution of cell populations towards irreversible malignancy, they cannot capture reversible drug resistance, local regulations nor epigenetic adaptations. We refer to the book [151] for their use in general biological settings.

Evolutionary game theory models [14, 15, 16, 166, 167] are another type of probabilistic model that can represent evolution in cancer cell populations, both with and without treatments. In evolutionary game theory, cancer cell phenotypes are the players of the game, their choices of interaction with other phenotypes are their strategies, and pay-offs refer to the fitness gain or loss from such interactions. The model calculates the relative abundance of phenotypes through time and selection corresponds to the most abundant phenotype at equilibrium (a so-called "evolutionary stable strategy"). However, evolutionary game theory models are restricted to considering a finite number of phenotypes and are limited in their ability to capture cell-environment interactions. Despite this, they are useful in teasing apart the intra-tumour interactions that lead to the relative abundance and coexistence of distinct phenotypic variants [149].

ODE models and heterogeneity. Using non physiologically structured differential models, *i.e.*, ODE models, makes it compulsory to divide the cell population into a finite number of compartments, in each of which the phenotype representing biological variability is a constant parameter, see an example in Fig. 4.2.

$$\begin{cases} \dfrac{dx}{dt} = xf(y) + \alpha f(y)(y-x), \\ \dfrac{dy}{dt} = yf(y) - u(t)g(y-x), \\ \dfrac{dn}{dt} = nf_1(n) - u(t)g_1(n), \\ x(0) = x_0, \quad y(0) = y_0, \quad n(0) = n_0. \end{cases}$$

Figure 4.2: *Example of a compartmental ODE model [54] of a growing cell population divided into 3 subpopulations: cancer drug-resistant (x), total cancer (y) and normal (n); $f(y)$ (respectively $f_1(n)$) stands for the instantaneous growth rate of the total cancer population (y) – the same as for the resistant cancer subpopulation –, (respectively for the normal population (n)), exchanges from the drug-sensitive ($y - x$) towards the resistant compartment (x) are represented by a fixed rate α, $u(t)$ represents an instanteneous anticancer drug delivery flow, $g(y - x)$ and $g_1(n)$ are drug sensitivity functions in cancer and normal cell subpopulations, respectively. This ODE model, published in 1997 by Michel Iskin da Silveira Costa and José Luíz Boldrini, was likely the first used to study optimal control strategies (see below) taking into account both resistance in cancer cell populations and unwanted side effects in healthy cell populations as a toxicity constraint, with the objective to minimise the total cancer cell population.*

A recent review of classic ODE models of tumour growth and their experimental assessment is [24]. Such a modelling setting has proved interesting to study the impact of a change of parameters on the whole cell population. This has been the case, for instance of [240] in which the effect of an increase of self-renewal on the growth of a leukaemic clone is studied, of [185] in which the glucose-lactate metabolism is studied, especially in glioma cell populations, and of [99] in which a non-local Lotka-Volterra ODE model is the basic set of equations proposed to represent between-cell population interactions in a tumour micro-environment. This latter model, as other models of evolutionary game theory (see above), may be considered as variants of the replicator equation [124, 197], that has been extensively studied in mathematical ecology.

Integro-differential (IDE) models. IDE models are structured in a phenotype variable that describes heterogeneity in a continuous way, but as regards differential terms, they differ from ODE models only in as much as an integral term represent-

ing non local interactions (*i.e.*, of one cell with all the others in the population, whatever their phenotype) is present. Recently, modelling techniques borrowed from mathematical ecology [66, 65] have been used to study the adaptation of cancer cell populations to the deleterious effects of anti-cancer therapy by using such models. Rather than considering two cancer-cell types that are either drug-sensitive or drug-resistant, instead in these models cancer cells are assumed to reside within a continuous spectrum of phenotypic states ranging from complete sensitivity to complete resistance to the therapy. In this framework, a continuous variable encapsulates the level of drug resistance of a given cell, and could be related for observation purposes in experimental settings to a specific molecular mechanism of cellular resistance (such as the expression levels of ABC transporter family proteins which can pump a variety of drugs out of cells [107]), or plainly to the minimum dose of a drug that kills a given percentage of the cell population in a controlled environment, but also, simultaneously assessing plasticity in individual cells, to stem cell membrane markers. Furthermore, the distribution of a phenotypic trait across a cell population evolves in time according to IDEs which incorporate both population dynamics (evolution with time of the number of cells disregarding their phenotypes) and adaptive dynamics (evolution with time of the probability distribution of the phenotypic trait in the population). See an illustration in Fig. 4.3.

The model proposed in [161] used such an approach to investigate how intratumour heterogeneity affects multi-drug resistance. The cancer cell population was assumed to be well-mixed with uniform exposure to a cytotoxic drug, and the level of drug resistance of a cell in the model was assumed to undergo small changes between cell generations through, for instance, genetic or epigenetic mechanisms. In the model, the level of resistance and the total size of the cell population determined the net growth rate of cells during therapy. Numerical simulations of the model equation indicated that therapy acts as a selection process causing the expansion of resistant clones. The phenotype-alteration process, on the other hand, was found to act as a diffusion process in the phenotypic space, effectively increasing stable heterogeneity and the likelihood of the presence of resistant clones that can survive therapy. The authors thus proposed that treatment which reduces phenotype alteration rates may improve targeted therapy.

In [175], the authors considered a model with a structure similar to that mentioned above [161] with the addition of an extra population of healthy cells that compete with the cancer cells for space and resources and are also exposed to the therapy. In this way, the model is able to incorporate the natural idea (mentioned below about therapeutic optimisation) that a therapy should exploit dynamic dif-

$$\frac{\partial}{\partial t} n_H(x,t) = \left[\frac{r_H(x)}{1 + k_H u_2(t)} - d_H(x) I_H(t) - u_1(t) \mu_H(x) \right] n_H(x,t),$$
$$\frac{\partial}{\partial t} n_C(x,t) = \left[\frac{r_C(x)}{1 + k_C u_2(t)} - d_C(x) I_C(t) - u_1(t) \mu_C(x) \right] n_C(x,t),$$

Environment: $I_H(t) = a_{HH} \cdot \rho_H(t) + a_{HC} \cdot \rho_C(t), \quad I_C(t) = a_{CC} \cdot \rho_C(t) + a_{CH} \cdot \rho_H(t),$

with $\rho_H(t) = \int_0^1 n_H(x,t)dx, \quad \rho_C(t) = \int_0^1 n_C(x,t)dx,$

u_1 cytotoxic drug, $\quad u_2$ cytostatic drug.

Figure 4.3: *Example of an Integro-Differential Equation (IDE) nonlocal Lotka-Volterra model with drug resistance phenotype structure [175]. In this IDE model, the two cell populations, healthy (n_H) and cancer (n_C), are represented with a quantitative structure trait x, standing for the expression of a drug resistance phenotype that impinges on both the intrinsic proliferation rates $r_H(x)$ and $r_C(x)$, on the death rates $d_H(x)$ and $d_C(x)$ and on the drug sensitivity functions $\mu_H(x)$ and $\mu_C(x)$ (in numerical simulations: weakly on healthy cells and strongly on cancer cells). The two cell populations interact only by competing - in a non-local way, each cell with all the others - for space and nutrients, as can be seen on the logistic terms $d_H(x).I_H(t)$ and $d_C(x).I_C(t)$, however with no direct confrontation (no bilinear law-of-mass-action-like encounter term). This model is amenable to optimal control strategies for the combined delivery of the two anticancer drugs (see below Fig. 5.1 and reference [209]).*

ferences between healthy and cancer cell populations. A combination of two therapies was considered: a cytotoxic drug which acts to increase the death rate of all cells according to their drug-resistance levels, and a cytostatic drug, which reduces the proliferation rate of all cells but preferentially affects cancer cells. Also, a cost was assumed to be associated with adopting increased levels of cytotoxic-drug resistance so that, in the absence of therapy, cells more sensitive to therapy are selected, whilst during therapy they are not. Numerical results of this model show that it is possible to drive the cancer cells to extinction and maintain a finite population of healthy cells only for certain combinations of drugs. Therefore, the authors propose that an important aim of anti-cancer therapy, to avoid the selection of resistant clones, should be to optimise drug doses rather than applying the so-called maximum tolerated dose (MTD), which is in accordance with the

principle of metronomic therapy [199].

Simple first-order PDEs: McKendrick-like transport equations. Transport equations are PDEs with only first-order differential terms. They have been extensively studied in their applications to biology in [201]. One of the most famous transport models is the McKendrick equation for the cell division cycle [182], in which the structure variable is age in the cell cycle, on which the focus is thus set to represent the relevant heterogeneity in the cell population. This modelling frame has been used in many other settings (*e.g.*, [4, 21, 95, 30, 201]), to represent cell population growth by progression in the cell cycle in a population of cells, tumour or healthy, but never thus far, to our knowledge, to study evolution towards drug resistance.

$$\frac{\partial n}{\partial t}(x,y,t) + \underbrace{\frac{\partial}{\partial y}\Big(v(x,c(t);\bar{v})n(x,y,t)\Big)}_{\substack{\text{stress-induced adaptation}\\ \text{of the proliferation level}}} = \underbrace{\Big[p(x,y,\varrho(t)) - d(x,c(t))\Big]n(x,y,t)}_{\text{selection}} + \underbrace{\beta\Delta n(x,y,t)}_{\substack{\text{non-genetic}\\ \text{phenotype instability}}},$$

with $\varrho(t) = \int_0^1 \int_0^1 n(x,y,t)dxdy$.

Figure 4.4: *IDE-PDE model with 2D- phenotype structure [48] to represent reversible drug resistance in a cancer cell population exposed to massive drug doses, as reported in [231]. Here, $n(x,y,t)$ is the population density of cells with phenotypic expression (x,y) at time t. The drug resistance phenotype is 2-dimensional, x standing for a potential of survival in extreme conditions (possibly linked to a plasticity capacity of a small number of cells) and y standing for a proliferation potential. The 2D nature of the drug resistance phenotype was suggested by the observations reported in [231] (see also Fig. 4.5). The Darwinian selection term has the same nonlocal Lotka-Volterra-like structure as shown on Fig. 4.3, except that here only cancer cells are present. It is shown on simulations in [48] that the non-genetic phenotype instability term is mandatory to obtain evolution from sensitive (PC9) to established resistant (DTEP) cells, whereas the stress-induced adaptation, 'Lamarckian-like' term is dispensable, provided that resistant cells are already present in the initial population, but mandatory if there are none.*

IDE-PDE models. When one wants to include instability in the structure variable (*e.g.*, a phenotypic trait), one may do so by using an integral term with a mutation-

like kernel as in [175], but one may also use a second-order term (a Laplacian) with respect to the structure variable (when the structure variable is space, instability is called diifusion), and optionally a first-order one (gradient or advection term) to represent fast evolution with respect to the structure variable [202].

In the IDE models discussed above [161, 175], the spatial structure of the cancer cell population is ignored and all cells are assumed to be equally exposed to the therapy. However, chemotherapeutic drugs often have poor penetration into solid tumours for a variety of reasons including the physico-chemical properties of the drug and the complex tumour microenvironment [189]. Nutrient and metabolite concentrations also vary across solid tumours [3], creating distinct niches that may favour different cell clones and hence diversification within a solid tumour. Therefore, to better understand the principles underlying how intratumour heterogeneity affects the emergence of drug resistance in solid tumours, the spatial arrangement of cells should be included in models. Motivated by these considerations, in the next generation [174] of the model proposed in [175], the authors incorporated an additional structuring variable to represent the spatial positions of cells inside a radially symmetric tumour spheroid. Only cancer cells were modelled. However, as with the previous model, the response of the cancer cell population to a combination of cytotoxic and cytostatic drugs was considered, although in this case the drug infusion was modelled as a diffusion process from the external boundary of the tumour towards its interior. The concentration of a resource that is assumed to be necessary for cell proliferation was also modelled in this way. Extra insight gained by adding space structure in the model included that it is possible for heterogeneity in cytotoxic-drug resistance levels to be present across the tumour both with and without therapy: a gradient of increasing drug sensitivity towards the boundary of the tumour was found without therapy, while during cytotoxic drug therapy this gradient was reversed so that more drug-resistant clones were found at the exterior (confirming in this model setting that high drug doses decrease heterogeneity in cancer cell populations [106]). Interestingly, when cytostatic drugs were infused in both of these systems (with and without cytotoxic drugs), the selection gradient along the radius of the tumour become less steep.

The IDE-PDE framework is also amenable to study the emergence of reversible drug tolerance in cancer cells populations. For instance, in [48], see Fig. 4.4, this framework was used to study an *in vitro* system where the emergence and subsequent reversal of drug-tolerance was observed during the administration and following the washout of high-doses of chemotherapeutic drugs [231]. Instead of structuring the cell population according to the level of drug-resistance (which is usually considered to be irreversible), this time the population was struc-

tured by a phenotypic trait variable related to the survival potential of cells during therapy (which can be reversed). As the average proliferation rate of surviving cells was observed to change throughout the *in vitro* experiments, an additional structuring variable was incorporated into the model to represent the proliferative potential of cells. Both phenotypic variables were allowed to vary, and in this case the rate of phenotypic variation was not limited by the cell proliferation rate since variation was assumed to be non-genetic by nature, and therefore not highly correlated to cell division events. The inclusion of the extra phenotypic variable meant that the model was able to capture the observed experimental dynamics whereby the proliferative but drug sensitive cells were transiently replaced by a small population of slowly proliferating and drug tolerant variants during therapy that then changed their phenotype to resume normal proliferation (still in the constant presence of the drug) and repopulate the sample. Furthermore, the appearance of a slowly proliferating cell population during therapy was found to be dependent on administering a high enough drug dose; when low-dose therapy was administered during *in silico* experiments, the average proliferation rate of the surviving cell population failed to drop during the experiment which is suggestive of an inherent risk of chemotherapy failure if the drug dose is too low. This first study [48] has been followed by others using the same principles, studying particular aspects of the initial model [47, 172].

ABMs to represent heterogeneity. In its simplest form, modelling drug resistance levels in a cancer cell population has been proposed as an "all or nothing" trait such that cells are classified as either completely sensitive to the drug or wholly resistant. This was the approach used in particular by the authors in [255] to investigate the role of short-range cell dispersal in the progression of metastatic legions and primary tumours. When considering dispersal, it is natural to consider spatial variables and in this paper the authors structured their cell population according to their position on a three-dimensional lattice. In this ABM, cancer cells proliferate at a rate proportional to the number of empty neighbouring lattice sites and die at a constant rate. Hence, cells proliferate more often near the edge of the tumour since there are more sites empty of cancer cells. If a cancer cell proliferates, one offspring cell moves with a small probability M to a nearby space on the lattice and there is a small probability that the progeny will mutate. Simulations of the model showed that when driver mutations were linked to resistance to anti-cancer treatment, the capacity of cells to move over short distances was found to impact on the regrowth rate of tumours following cessation of the therapy. This insight into carcinogenesis was captured by the spatial model. However, an assumption

of the model is that the drug concentration was distributed uniformly throughout the tumour which is unlikely, as mentioned above [189]. Therefore, incorporating between-cell heterogeneity in exposure to the drug may influence these results.

Another way to represent heterogeneity in cancer cell populations in agent-based models (ABMs) is with a continuous variable. The model [48] shown in Fig. 4.5 did exactly this by using a two-dimensional phenotypic trait describing the survival potential (likely corresponding to a high plasticity of the concerned tumour cells, making them able to adapt to extremely hostile conditions such as massive doses of a cytotoxic drug) and the proliferation potential of cells. These continuous traits influenced the proliferation and death rates of individual cells and were made to fluctuate in time in each cell according to a discretised set of stochastic differential equations. The model was formulated to describe the population dynamics and phenotype evolution of a cell population exposed to a drug insult in a Petri dish. It was inspired by the striking results on reversibility of drug resistance reported in [231], and could qualitatively reproduce these experimental results.

Hybrid models. Hybrid models offer a more complex approach to modelling heterogeneity and drug resistance in cancer by coupling models which are deterministic in one sense and probabilistic in another. For instance, hybrid models may describe cells as discrete entities that can proliferate, die and migrate according to probabilities, and couple this process to a continuum description of the distribution of diffusing nutrients and metabolites in space, which may be influenced by, and influence, the metabolism of cells. These types of hybrid models are particularly useful when considering the evolutionary feedback between tumour cells and their micro-environment .

These ideas were explored by the authors in [214] who were interested in how intra-tumour metabolic heterogeneity impacts treatment outcomes. In this study, a hybrid cellular automaton mathematical model in two spatial dimensions was used to model the evolution of a tumour and its micro-environment: individual cells were modelled as discrete agents in a two dimensional lattice, while reaction-diffusion equations modelled the distribution of oxygen, glucose and protons. Two continuous variables, not related to drug resistance, but instead to metabolism preference and resistance to acidity characterised individual cells. These traits were assumed to undergo small variations during proliferation, and influence the rate of tumour cell death, proliferation, proton production and oxygen and glucose consumption. Simulations of the model reveal predictable patterns of cellular phenotypes along the radial dimension of the tumour that change in time and are

perturbed during therapy. Based on insights gained from repeated simulations of the model, the authors proposed that early treatment with cytotoxic therapy causes a subtle restructuring of the spatial heterogeneity of phenotypes which essentially limits the ability of more aggressive cells to invade. However, later treatment more effectively strips away a layer of less aggressive cells that initiates fast tumour growth. In this case, the hybrid model revealed that some instances of resistance to anticancer therapies may be related to the evolving architecture of the tumour.

Another class of hybrid models are the piecewise deterministic Markov processes (PDMPs) [61], which might be used to represent continuous in time, deterministic – and reversible, *i.e.*, in particular amenable to the representation of epigenetic modifications – cell population models of cell proliferation separated by stochastic jumps representing irreversible driver mutations in the DNA; to our best knowledge, although PDMPs are an active field in mathematical biology, this still remains to be done. The basic deterministic dynamics in this scenario could be a hidden phenotype-structured cell population PDE model with bistability and hysteresis (as is, *e.g.*, PU.1/GATA1 in haematopoiesis [46, 111, 136]) to represent evolution of the cell population according to a manifest phenotype (in this instance, red or white blood cells). Forsaking bistability (hence at the cell-population level stochastic balance according to random choice of initial conditions), *e.g.*, due to change in environmental conditions leading to a saddle-node bifurcation [87], would lead to an unbalanced cell fate. The overwhelming choice of one fate leading to non sustainable dynamics on the long term, makes it more probable that an irreversible jump from the deterministic and reversible dynamics of the cell population occurs. In such a PDMP, the probability of a jump, of course (since it is Markovian) cannot depend on the previous jump, but should depend on the state of the intermediate deterministic model describing the between-jump population dynamics.

5. Perspective: possible consequences for therapeutic optimisation

In light of this review of cancer models, how can we represent relevant targets for drugs, and how can we design optimised therapeutic strategies to control these targets [27]? Should we have as an objective the complete eradication of cancer cells? Or, remembering that they are very good at proliferation and survival in extreme conditions, and that for them "what does not kill me strengthens me" [203], should we not, on the contrary, forsake the pursuit of this eradication goal and drive the targeted cancer cell population away from acute, uncontrollable proliferation and establish cancer as a chronic disease? With this view, the

aim would be to contain it within limits compatible with the patients quality of life. Should treatments thus be tailored to allow survival of "nicer" cancer cells, maintaining on the long run such situations by"adaptive therapy", as proposed by Robert Gatenby [96, 97, 98]?

These are some of the questions control scientists are confronted with. In control science, a dynamical system represents an evolving phenomenon that mathematicians or engineers want to keep within prescribed limits (control) or lead to a desired endpoint (optimal control) by exerting external means that have known effects on known targets in some of its constituents. In clinical control of tissue growth, drugs are the main means of action, their functional targets are proliferation, death and differentiation rates, and cell populations should be kept under control or sent to a desired equilibrium point, with either zero cancer cell, or, more realistically, with a very small population of them, with little impact on the quality and number of healthy cells.

5.1. Exploiting structural differences between healthy and cancer cells

In designing optimal strategies to deliver anticancer drugs, one can just consider the simplified objective of eradication to be obtained in finite time as the only crucial goal and consider unwanted toxic side effects as mere levels not to be trespassed in average, on the basis of a daily dose, or of absolute limits never to be trespassed at any moment. Such limits are in these cases supposed to be granted by clinical knowledge, so that targetting cancer cells to kill as many of them as possible within these limits is the goal to pursue. To proceed this way and provide solutions to therapeutists is completely dependent on the versatile clinical habits of a given epoch; optimisation strategies can sometimes do better than that. Indeed, healthy cell populations that are such unwanted toxicity targets are for most of them (not all: cardiac toxicity, peripheral neuropathy, for instance, are exceptions) constituents of fast renewing tissues, such as haematopoietic bone marrow, intestinal mucosa, liver, skin, the proliferation of which can be modelled in the same way as for tumour tissues.

Then the theoretical therapeutic question: "how to optimise anticancer drug delivery to a given patient?" becomes a problem with two populations, healthy and cancer, that are partly proliferating, partly dying, both evolving under the same drug insult, however with structural dynamic differences between them. This question has been the object of several studies [17, 27, 30, 50, 51, 52], taking into account for some of them heterogeneity with respect to age phases in the cell division cycle [27, 28, 29, 30], but so far heterogeneity with respect to phenotypes determining drug resistance had only been sketched as prospective work [27, 51].

The latter has begun to be tackled using phenotype-structured models [174, 175] and optimal control strategies are presently under study (the structural differences between the two populations are related to different behaviours of the proliferation and death rates with respect to the structure phenotype [209]).

5.2. Ordinary differential equations and delay-differential models

History. Optimal control methods applied to overcoming drug resistance in cancer has a long history, that dates back at least to 1992 with Michel Iskin da Silveira Costa and José Luíz Boldrini [33, 54, 55, 56], who in a series of mathematical papers used two cancer cell populations, one sensitive and another resistant to a given drug therapy (see Fig. 4.2). These studies remained theoretical and do not seem to have used a physiological tumour environment in their representations.

Ordinary differential equations and optimal control strategies using drug combinations. More recently, Urszula Ledzewicz and Heinz Schättler have tackled the problem of combining two classes of anti-cancer drugs, one hitting the tumour cell population directly, and another one indirectly, choking it by an antiangionetic effect on its vascular environment, see, *e.g.*, [163]. Furthermore, the effects of metronomic therapy may be compared with those of the more classic maximal tolerated dose (MTD) [23] still in use in most clinical oncology departments [229]. These studies, based on the Hahnfeldt model [116] or some of its variants, that include both the tumour cell population and its environment considered as the tumour carrying capacity as a target for antiangiogenic drugs, have the remarkable feature to propose exact optimal solutions when it is possible. These authors do not consider so far the question of drug resistance nor the question of intra-tumour heterogeneity (see however recent developments modelling metronomic strategies applied to circumventing drug resistance in vitro in [43]). Nevertheless, they show the feasibility of such theoretical methods and they are concerned with applicability in the clinic [162], which opens the way to actually innovative therapies.

Optimal control of acute myeloid leukaemia (AML). Acute myeloid leukaemia is a cancerous disease that has raised the interest of mathematicians for some time already, with representations of its dynamics based on transport equations physiologically structured by age in the cell cycle [4, 196], with possible transformation into delay-dfifferential systems by integration along characteristic curves. On these grounds, Xavier Dupuis has proposed a theoretical solution to the problem of controlling proliferation of the leukaemic cell population by an optimal combination of cytotoxic and antiproliferative drugs [75], however, like the previous authors, not tackling the question of drug resistance.

5.3. Physiologically structured equations with drug resistance: open questions

Drug resistance in phenotype-structured equations. The IDE and PDE models mentioned above [47, 48, 161, 172, 173, 174, 175] are recent and have all explicitly been designed to predict the development of resistance, starting from models coming from mathematical ecology (see above) transposed to cancer. Of note, in these models, resistance is in principle always reversible and thus corresponds to epigenetic mechanisms of resistance, not to mutations. Including models with driver mutations that are certainly encountered in cancer would need using PDMPs (see above about hybrid models).

Optimal control in phenotype-structured models with drug resistance. Nevertheless, it is precisely in such situations, when drugs induce drug resistance, usually with an effect all the more so imprtant as the drug dose is higher, that optimal control strategies can be efficient, to avoid the constitution of genetically established drug resistance. As mentioned above in the section "Evolutionary mechanisms that contribute to heterogeneity in cancer", epigenetic modifications of the DNA are likely to occur prior to mutations at the single-cell level, and it is precisely this process that is represented at the cell population level by these phenotype-structured models. Models that are presently under study [209], see Fig. 5.1, pursue the goal to avoid or limit as much as possible such evolution towards drug resistance using a combination of cytotoxic and cytostatic drugs. Cytotoxic are the ones that are life-threatening for the cancer cell populations and that stimulate their plasticity to yield a subpopulation of resistant cells (observed "drug tolerant persisters") that will not be killed by the drug (and, as quoted from Friedrich Nietzsche's *Twilight of Idols* in [203], for a robust cancer cell population, "what does not kill me makes me stronger"[3]), whereas cytostatic drugs – at least at low or medium doses – are supposed to slow down proliferation but not to stimulate these population mechanisms based on some bet hedging of plasticity (a fundamental difference with healthy cells, that are far from plastic) that allow these cell populations to survive as such (a tiny amount of it is sufficient for this) in hostile conditions. Adding epigenetic drugs that would not only cease to favour this resistance process, but even more would be able to stop it (as KDM5A inhibitors in the experiments reported in [231]) would make such strategy more efficient; however epigenetic drugs are not easy to handle as yet [11, 121].

[3]The exact phrase, often quoted in many occurrences (yet certainly more often about men than about cancer cell populations) is found in Nietzsche's *Götterdämmerung, Sprüche und Pfeile 8: "Aus der Kriegsschule des Lebens. – Was mich nich umbringt macht mich stärker."*

Open problems in optimal control. The relevance of the use of metronomic therapy as an alternative to maximum tolerated dose of cytotoxic drugs in chronic forms of cancer is a question that has been actively studied for some time [23, 199], and more recently with optimal control methods [229]. Its achievements were firstly attributed to angiogenic effects of cytotoxic drugs [154], then to effects of low doses of chemotherapy on stimulating the immune response [199], in particular due to the effects of tumour lysates on the participation of memory T-cells in the immune response [265]. We also propose that avoiding the stimulation of plasticity mechanisms in tumour cells by avoiding as long as possible the delivery of high doses of cytotoxic drugs, and keeping the tumour cell population under check by cytostatic drugs, is another guideline to keep, and we are exploring optimal theoretical strategies strategies to do so [209]. Combining such strategies with optimisation of recently developed immunotherapies seems to be a therapeutic way for the future. Another challenge is, when robust resistant subclones in a cell population have been organised in finite number as a bet hedging (purely stochastic) strategy by the tumour, as this often seems to be the case, what can be done to identify these clones and eliminate them sequentially, taking advantage of differences in proliferation potential and plasticity/survival potential? Even better, is it possible to avoid by using epigenetic drugs the emergence of such robust isolates at a time when resistance phenotypes are still in a plastic, not fixed, state in the cell population? Solving this question should require the integrated skills of cancer biologists, pharmacologists and mathematicians.

6. Conclusion

We have tried in this review, from the point of view of mathematical modellers, to present together to the best of our knowledge past and more recent relevant works in cancer biology, evolutionary biology, mathematical modelling and optimal control methods to understand the role of between-cell heterogeneity in the emergence of drug resistance in cancer cell populations and to circumvent it. We have underlined the necessity for this purpose to take into account evolutionary effects, both in the genetic sense of Darwinian evolution and in the sense of modifications, reversible or not, of the epigenetic landscape attached to every single genome. Many open questions remain. In particular one can hope that unravelling the physical environmental history together with the phylogeny of evolved multicellularity will tell us more on possible catastrophic chained events that, from hardly perceptible epigenetic modifications may lead to established cancers, and by what means we could correct the course of such events. In other words, quot-

ing Paul Davies and Charles Lineweaver in their seminal essay [59], *"Rather than attacking tumors indiscriminately (the only good cancer cells are dead cancer cells), understanding their origin, managing them and containing them might be a far smarter strategy"*. In the same way, at the level of a constituted evolved multicellular organism (a patient), if we can have access to the main differentiating bifurcations in its epigenetic landscape and to the epigenetic factors that control them [111], we may, by taking advantage of the most recent knowledge about the evolution of cancer cell populations under the influence of their environment and possibly of new epigenetic drugs aiming to control (de)differentiation processes, design theoretical therapeutic strategies relying on optimal control methods that should in the not so distant future find their application in the clinic of cancers.

Authorship

Rebecca H. Chisholm and Jean Clairambault equally contributed to the writing, editing and revising. Tommaso Lorenzi contributed in the editing and final revision of the manuscript.

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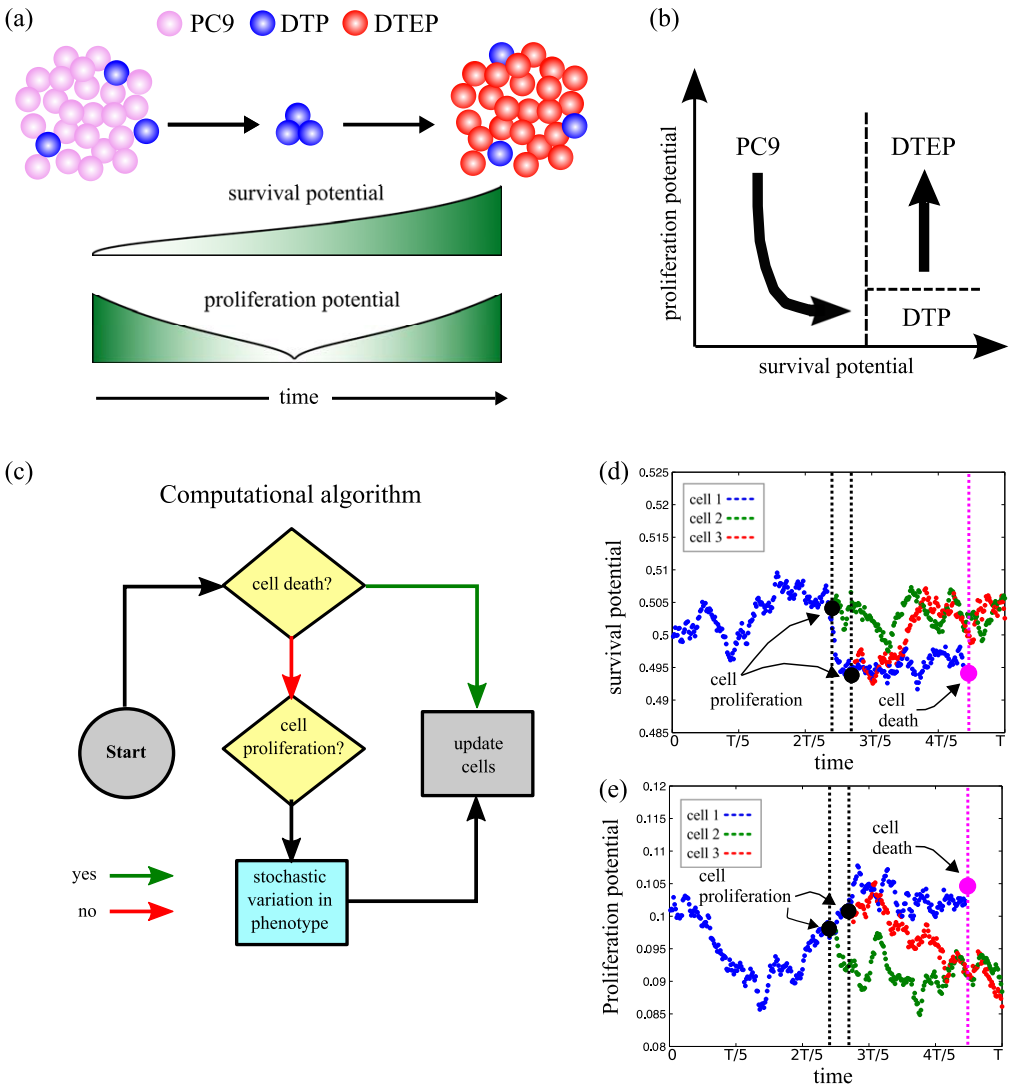

Figure 4.5: *An Agent-Based Model [48] designed to represent the same biological phenomenon mentioned in Fig. 4.4.* (a) *The drug resistance phenomenon reported in [231]: evolution from sensitive (PC9) to surviving but non proliferating (DTP) and finally to surviving and proliferating (DTEP) cells. The ABM representation, as the IDE-PDE one shown on Fig. 4.4, is compatible with the observed total reversibility of sensitivity when the drug is washed out.* (b) *Evolution of the cancer cell population in the* (x, y) *phenotype phase plane.* (c) *The computational algorithm, in which non genetic instability is represented by stochastic variation in the phenotype* (x, y). (d) *Three different simulations of individual cell fates with followup of the survival potential* x *and of the proliferation potential* y. *The averages of these trajectories on large numbers of stochastic simulations recover the deterministic trajectories of the IDE-PDE model, as shown in [48].*

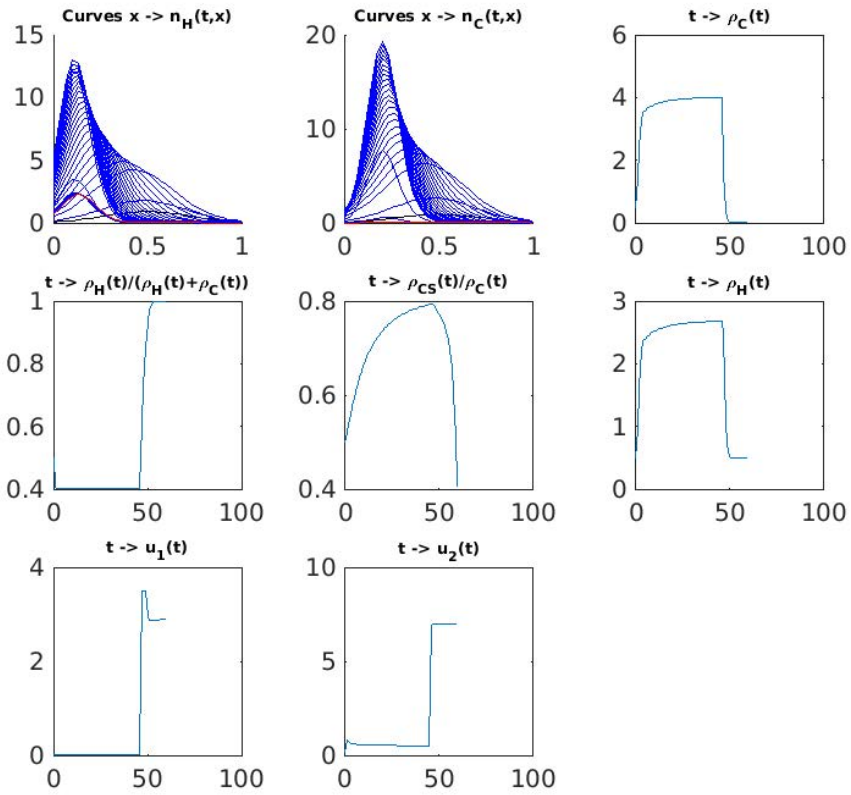

Figure 5.1: *Result of an optimal control strategy for the combined drug delivery flow of two anticancer drugs, one cytotoxic, $u_1(t)$, and one cytostatic, $u_2(t)$, control performed on the IDE model shown on Fig. 4.3. Cytotoxic drugs, e.g., alkylating agents such as temozolomide or platinum compounds, kill cells, whereas cytostatic drugs such as growth factor receptor antagonists - in particular the tyrosine kinase inhibitor gefitinib used in the first place at very high doses in the experiments reported in [231] - only slow down the cell division cycle without killing cells* provided that they are given at low doses. *In this respect, cytotoxic $u_1(t)$ and cytostatic $u_2(t)$ drugs could represent the same drug, at high and low dose, respectively. The optimal control strategy can be shown to be as presented on this figure, i.e., no cytotoxic as long as possible to avoid the development of a resistant cancer cell subpopulation (a so-called "drug holiday" in clinical settings) but a moderate dose of cytostatic at the same time, and when the maximum of concentration of phenotypes has been reached, then maximum tolerated dose of both drugs to drastically decrease the cancer cell population $\rho_C(t)$ until a bottom healthy cell population $\rho_H(t)$ has been reached, and then stop cytotoxic drugs to avoid the development of a resistant cell population $\rho_C(t) - \rho_{CS}(t)$ in the likely event of non eradication of all cancer cells [209].*