Optimizing cancer pharmacotherapeutics using mathematical modeling and a systems biology approach

Research in mathematics and in mathematical biology on cancer and its treatments has been soaring in the past 10 years at an unprecedented speed. Such thriving is likely due as much to new findings in fundamental biology as to an emerging general interest from mathematicians and engineers towards applications in biology and medicine and to their subsequently designed representations and predictions of tumor processes that are now allowed by modern means of computation and simulation. This article, which does not claim the status of an extended review paper on mathematical models of cancer and its treatment, is focused on modeling in a systems biology perspective. I will list here the most necessary mathematical methods, in my opinion, which, while enforcing already existing methods, should be further developed towards designing and applying optimized individualized treatments of cancer in the clinic.

KEYWORDS: cancer ▪ mathematical models ▪ pharmacokinetics–pharmacodynamics ▪ systems biology ▪ therapeutic optimization ▪ treatment individualization

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Mechanistic & physiological modeling in systems biology for cancer therapeutics

I will show in this article that existing mathematical methods should be combined to yield a rationale for optimizing the treatments of cancers. They rely on three different approaches:

- Physiologically structured models of cell population growth based on a representation of the cell cycle and its physiological and pharmacological controls (structured population dynamic models: the target);
- Whole-body physiologically based pharmacokinetic–pharmacodynamic (PK–PD) models for anticancer drugs, from their input in the general circulation to their output on cell and tissue proliferation (the means of action in the clinic);
- Numerical optimization methods for the delivery of drugs under the constraints of limitation of unwanted toxic side effects and of emergence of drug-resistant tumor cell clones (the theoretical methods to provide clinicians with a rationale for optimizing treatments of cancers).

Growing interest of mathematicians & engineers towards biology & medicine

Among organizations of engineers, such as the Institute of Electrical and Electronics Engineers (IEEE) or International Federation of Automatic Control (IFAC), more and more room is dedicated in international conferences to biomedical modeling, with a focus on cancer. More recently, societies and networks of mathematicians (Society for Mathematical Biology [SMB], European Society for Mathematical and Theoretical Biology [ESMTB]) have emerged, organizing conferences and summer schools on mathematics applied to cancer modeling and therapy. Even more recently, integrated cancer centers have appeared that hire physicians, biologists, mathematicians, chemists, physicists and computer scientists working together in specialized teams on subjects related to cancer with medical applications. Numerous recent publications testify to this interest among applied mathematicians [1,2].

Pharmacology of cancer, variability in drug response & personalized medicine

Cancer in all of its forms has become the primary cause of death in several industrialized countries, including France [3,101] and the USA [4], when all categories are merged. It potentially puts cell integrity of all tissues in the organism at stake as a result of the deficiency of various control mechanisms. The complexity of this disease may help to explain the difficulties met in achieving major therapeutic advances in oncology and the resulting partial stagnation of the number of deaths due to cancers in the last quarter of a century, whereas at the same time the number of deaths due to cardiovascular diseases decreased by 50% [3,4]. In the challenging context of this

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limited therapeutic success, personalized medicine should not be neglected: by merging subjects amenable to an adapted treatment within distinct categories, defined by their common response toward a given drug, one may expect global improvements in the population response to existing anticancer therapies through a better handling of drug delivery to individual patients.

Indeed, we are not constitutively (in particular genetically) equal in terms of our reaction to diseases and the responses of our bodies to medical treatments. Cell mechanisms, mainly enzymatic, which activate, degrade or expel (using energy-consuming molecular pumps) absorbed drugs, do vary from one subject to the next (between-subject variability), which in principle imposes adaptation to every single patient dose and administration means. Such molecular mechanisms can be represented at different scales, as sketched in Figure 1, by mathematical models that may be subsequently identified and individually quantified by tissue samples from patients to whom drugs will be delivered.

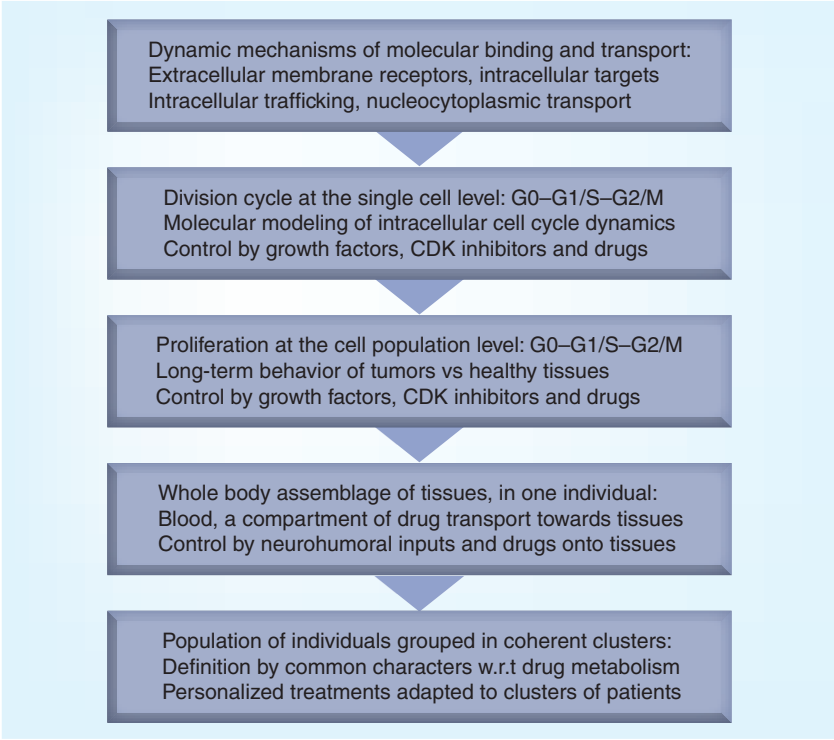

Figure 1. Multiscale spatial perspective for modeling, from molecules to populations of patients. Each triangle stands for a magnifying glass, from bottom to top. Drugs are present at each scale: prescribed at the lowest level of magnification, they act at the highest molecular levels. In this perspective, genes *per se* are not primordial. All that matters is their physiological meaning in terms of impact onto molecular mechanisms of cell division and drug effects. These effects may be dependent on several genes, as well as on epigenetic factors, and the most accurate elementary description level is hence more likely to be dynamic and molecular, both for the physiological control mechanisms of cell division and for cell drug metabolism.

This is of course easier said than done, because tissues are not always accessible and, furthermore, their responses to treatments may be different in cultures from what they would be in the whole organism, where local and central regulations are present. Nevertheless, at least a raw idea of the intracellular and intercellular regulation mechanisms may be obtained from *in vitro* studies, bearing in mind that straightforward transposition from *in vitro* to *in vivo* settings may be hazardous and at least requires taking into consideration higher level regulations.

First, to take into account between-subject variability, investigating genetic variations in intracellular processing of drugs may be of major importance. In this respect, in the field of another medical discipline, antibiotherapy, the case of isoniazid is historically well known among physicians: when it appeared as an anti-TB drug after World War II, it was noted that the same doses (for a given weight) of this new drug could be efficacious but very toxic in some subjects while in others it was neither toxic nor efficacious. The explanation of this puzzling phenomenon was shown to reside in the interindividual variations of the main enzyme that degrades isoniazid, an acetylase. The general population is genetically divided into two groups with respect to this enzyme: slow acetylators, among whom slow degradation of the drug results in efficient tissue concentrations at low doses, and fast acetylators, among whom fast degradation of the drug makes high doses necessary to achieve the same result [5,6]. This enzyme (*N*-acetyl transferase) thus shows striking genetic polymorphisms.

In clinical settings, taking into account genetic polymorphisms of cell drug processing is hence one of the important aspects of personalized medicine and this holds for cancer therapeutics, in which it has been shown to be important at least for some drugs, such as irinotecan [7–9], to better predict undesirable adverse effects. One should consider not only enzymatic degradation metabolism, which is seldom determined by only one gene, but also baseline concentrations of target molecules for the drugs and the function of active efflux transporters, the ATP-binding cassette transporters, which expel exogenous molecules from cells, using an ATP-driven pumping mechanism [8,9]. At the level of a population of patients, too seldom so far considered differences between males and females, which may be due in particular to differences with respect to drug processing mechanisms, result in differences of toxicity limitations in

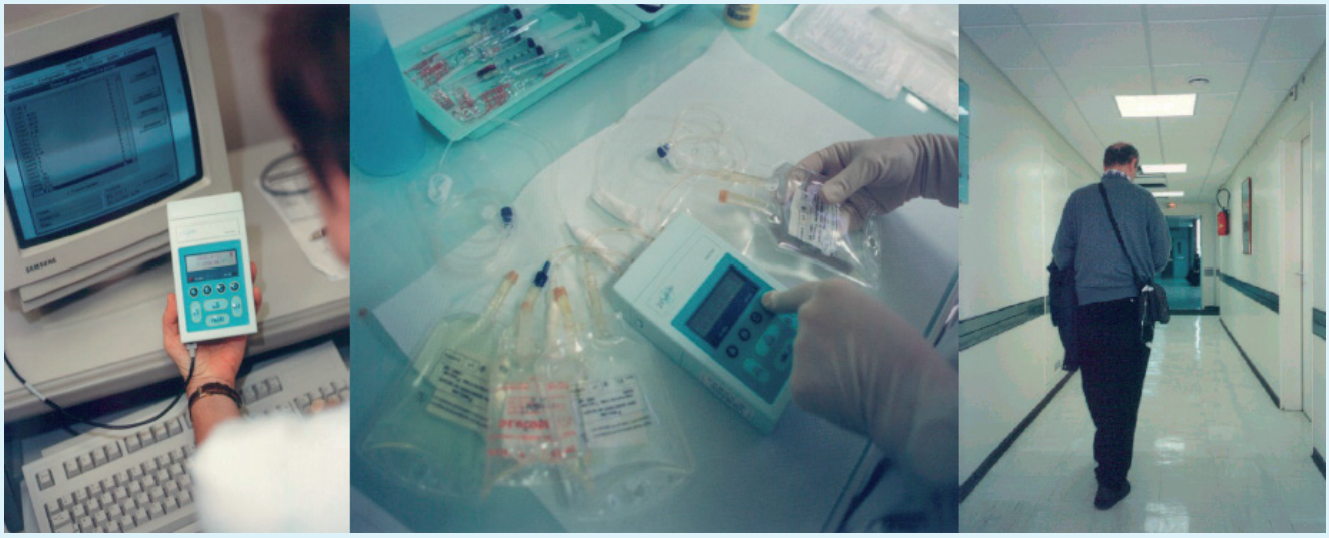

Figure 2. Portable programmable device for multidrug infusion (four channels), endowing the patient with a 3-week autonomy.
Reproduced with permission from [107].

anticancer treatments [10]. Going beyond drug processing enzymes, mutations impacting intracellular signaling pathways (e.g., involving protein KRAS), should also be considered. But, as has been evidenced by the limited success encountered so far in adapting drug treatments to only single-gene variations [6], considering not only the gene level, but also the physiological level of cell and tissue drug metabolism by the representation of dynamic parameters (e.g., enzymatic), may be more relevant in describing differences between individuals.

Second, apart from between-subject variability (nonetheless still taking it into account), as far as within-subject variability with respect to responses to drug treatments is concerned, an important source of such variability is the influence of molecular circadian clocks on both cell proliferation mechanisms and drug metabolism processes. Among other developments, circadian chronobiology has led to cancer 'chronotherapeutics' in the clinic, which is the discipline that determines the best time of day – the most efficient and the least toxic to healthy tissues – to administer a given treatment. Its practical clinical applications, in particular from the point of view of its technological implementation, involve the use of ambulatory programmable delivery pumps that can endow cancer patients with 3-week total autonomy (Figure 2) [10].

Circadian chronotherapeutic treatments of cancer have achieved successful results in the past 15 years, in particular for metastatic colorectal cancer, which have been partially explained. Most of the time these explanations involve the role of circadian rhythms in the activity of drug processing enzymes [10]. Patients with treated metastatic colorectal cancer and disrupted circadian rhythms show a marked decrease in overall survival, compared with the same tumor-bearing subjects with the same treatment, but with preserved rhythms ($p < 0.001$) [11].

In a whole-body spatial perspective, systems biology is used, in the context of cancer treatment in general, in at least two situations: to represent cell proliferation (by models of the cell division cycle in cell populations) and to represent the fate of drugs in the organism (systems pharmacology). In each case, the representation should be multiscale, from molecules to cells, tissues and the whole organism, for at least two reasons. First, because dividing cells are controlled by molecular mechanisms that must be modeled, not only at the single-cell level, but also at the cell population level for normal and for tumor growth and even at the level of the whole organism if one takes into account the necessary balance between antitumor therapeutic efficacy and unwanted toxicity to various healthy organs. Second, because drugs are given at the level of a whole organism, but move through the body, from compartment to compartment, to reach tissues and exert their actions in cells at the molecular level, which must be represented by intracellular PK and PD.

In the same way, in a temporal perspective, the mathematical modeling frame of differential equations is the best suited because cell and tissue

proliferation on the one hand and drug diffusion and effects in the organism on the other hand are both continuous (i.e., dynamic) processes. The need for such a rationale to help clinicians design improved anticancer treatments has emerged from regular collaborations between researchers and physicians who work both in experimental and in clinical settings, with the goal to rely less on trial and error in designing therapeutic schedules. Cancer chronotherapeutics makes the use of a dynamic modeling frame to represent the influence of circadian clocks on tissue proliferation and drug processing inevitable, but the use of differential equations goes way beyond chronotherapeutics, as is evidenced by recent pharmacological literature [12].

Mathematical models of cancer & its pharmacological treatments: a systems biology viewpoint

From mathematical models of proliferation to the proposal of optimized therapeutic strategies in the clinic, theoretical research with a therapeutic goal in oncology should develop according to the three main directions detailed in the introduction of this article (see also [13]), describing:

- The physiological system that is perturbed in cancer tissues: proliferation of cell populations, described not only in cancer tissues but also in fast-renewing healthy tissues, since drugs delivered in the general circulation seldom make a difference between healthy and cancer cells;
- The methods in the hands of oncologists: drugs and their fate from entry into the general circulation until their impact on their molecular targets, desired or not, with consequences for the control of cell proliferation, both healthy and tumor;
- Rational ways by which the delivery of drugs by clinicians may be scheduled so as not to hamper too much physiological proliferation in healthy tissues and also not to stimulate the emergence of drug-resistant cell clones in tumors, which are ways of theoretical therapeutic optimization.

The first of these directions is the representation of cell proliferation and its control by a description of the cell division cycle, both in health and in cancer, together with its control at the scale of a cell population, by physiologically structured partial differential equations (PDEs). In these model equations, which are intended to represent proliferation in a therapeutic perspective, the time evolution of the population does not – or not only – depend on space coordinates in the medium. Rather – since cancer therapeutics primarily act by impacting the cell division cycle – it depends on physiological variables such as concentration of cyclins or more globally on a lumped variable representing physiological age with respect to cell cycle phase timing. Such models may be linear, thus yielding only exponential behavior of the evolution with time of the cell populations they represent [14], but it is also possible to endow them with a nonlinear feedback representing growth limitations due to scarcity of space or nutrients [15,16]. The control targets are the cell cycle checkpoints which ensure genome integrity for the cells committed to the cycle. These checkpoints are physiologically determined by Cdks, their inhibitors and activators, as described at the single-cell level for instance in [17], from which can be extracted simplified models [18] or even simpler previous models by the same authors and used to yield representations of Cdks as control inputs to cell population models (functions K_{i-i+1} in Equation 1) [19].

$$\begin{cases} \frac{\partial}{\partial t}n_i(t,a)+\frac{\partial}{\partial a}(v_i[a]n_i[t,a])+(d_i+K_{i\to i+1}[t,a])n_i(t,a)=0, \\ v_i(0)n_i(t,a=0)=\int_{\alpha\geq 0} K_{i-1\to i}(t,\alpha)n_{i-1}(t,\alpha)d\alpha, \\ \quad 2\leq i\leq I \\ n_1(t,a=0)=2\int_{\alpha\geq 0} K_{I\to 1}(t,\alpha)n_I(t,a)d\alpha, \end{cases}$$

Equation 1

Equation 1 provides an illustration of physiologically structured proliferation models at the cell population level: the Von Förster–McKendrick model. n_i, the variables that are solutions of the equations, are cell numbers (or densities) in the ith phase of the cell cycle at time t and age-in-phase a, phase I being the last phase (mitosis); d_i and v_i are death and progression speed terms in phase i whereas the K_{i-i+1} terms represent transition functions between phases. All these terms are functions that depend on time and physiological age and can be blocked or enhanced by external control. The total number of cells at time t is the sum of cells over all ages and all phases [14].

The second direction, molecular PK–PD of anticancer drugs, describes by ordinary differential equations (ODEs) the fate in the organism of therapeutic molecules used in oncology, at the cell and whole-organism levels, from its entry into the general circulation until its ultimate cell effects, either on the DNA or on determinants of the cell cycle machinery. This complete

process, from the entry of a drug into the general circulation until its effects on molecular targets in cells, passing through molecular binding to proteins in blood, partial renal and hepatic clearance, transport from blood to interstitial peripheral tissues and from there to intracellular medium, should be described by chemical rules based on the law of mass action or Michaelian kinetics when applicable, in a systems biology perspective. Recall that according to a well-known mnemonic motto, "pharmacokinetics may be simply defined as what the body does to the drug" – and this includes not only variations of blood concentrations but also of tissue concentrations and intracellular drug processing – "as opposed to pharmacodynamics which may be defined as what the drug does to the body" [20] – that is to say, for cancer treatments, molecular modifications of intracellular targets and secondary effects on tissue proliferation. A physiological concern about the representation in the PK part of actual body compartments, intracellular and extracellular organ after organ, should be present here, if one wants to take into account variations in drug response in a population of patients. This cannot be obtained through a statistical shortcut between genes and individuals, but by taking advantage of any existing knowledge about the mechanisms that are damaged – or reinforced – when cell targets are hit by drugs and of the mechanisms involved in drug processing by enzymes and transporters. This cannot be obtained in a physiological systems biology perspective through compartmental modeling, but can only be defined by concentration curve fitting and the definition of sheer descriptive characteristics, such as drug half-lives and peak drug concentration. The PD part of this modeling is a mechanistic representation of intracellular drug metabolism and should include the description of the molecular signaling cascade, from DNA damage performed by cytotoxic drugs, its detection by sensor proteins, transmission to the protein p53, until subsequent repercussions (e.g., cell cycle arrest, DNA repair and apoptosis launching) on the proliferation model at the cell population level. Not all of these steps in molecular PK–PD models may always be observed and scarcity of available data often induces the use of shortcuts between some of them, while maintaining detailed molecular description levels between others, according to the clinical problems under consideration. Nevertheless, starting from physiological principles and using techniques of model reduction, from complex to simple, should be preferred to oversimplified models strictly based on the availability of data, which will soon become obsolete when more elaborate investigation techniques are available, such as those that should come from constant progress in intracellular imagery.

Last but not least, in the perspective of applications to the clinic, the goal of the third direction of research is to optimize the delivery of anticancer drugs by using optimization algorithms, designed with the aim to destroy as many tumor cells as possible under the constraint of limiting unwanted toxic side effects to healthy cells. To this toxicity constraint, which concerns healthy cells, one may also add, whenever relevant mechanisms are known, another constraint on the unwanted drug-induced emergence of drug resistance in cancer cells. Optimization under constraints applied to therapeutics is a mathematical formulation of the usual trade-off, not specific to oncology, between therapeutic efficacy and toxicity, which aims to identify the best drug delivery strategy in a given biological or clinical context that will not be detrimental to the patient's health through unwanted side effects. It is important to note that optimized therapeutic control on cell and tissue proliferation can seldom be called optimal control, in the sense of engineering, for optimal control usually means optimization – possibly in real time – of a projectile trajectory, which is here a programmed drug infusion schedule in a deterministic PK–PD representation, to reach a well-defined and attainable target. In the case of cancer therapies such a target can only be eradication of the tumors, which is not always possible, or at least their containment under a given proliferation threshold, which is not easy to define. Furthermore, clinical feedback inputs for the adaptation of dose schedules occur on a time scale that makes human adaptation more advisable than real-time automatic control. For these reasons, relating to cancer therapeutics, it is more modestly spoken of optimization than of optimal control.

Mathematical models of cell proliferation & its control

Mathematical or physical models, designed on physiological grounds, which describe cell and tissue proliferation, are many and have been partially reviewed elsewhere [13]. Some of them are designed to mimic macroscopic tumor growth in various physical conditions, but do not include molecular targets for drugs. In particular this is the case for agent-based models, which are stochastic, not deterministic, with the double

drawback of leading to computationally very expensive simulations, presently limited to approximately 10^6 cells in development and, more importantly, to images that may mimic the physical phenomena under study, but without anyone being able to analyze them from a mathematical point of view. This point is important, for scores of simulations with various sets of parameters will never replace a demonstration about the existence of a property or the feasibility of an optimization method. Nevertheless, agent-based models are interesting as guidelines to building deterministic models. Other models are deterministic and include molecular targets, but stick to a single cell, skipping the cell population perspective that is essential in the representation of controlled (in health) or uncontrolled (in cancer) tissue proliferation. I advocate, rather, physiologically structured PDE models to represent the cell cycle in cell populations because:

- They can be designed so as to include the molecular targets of drugs that act by damaging the DNA, resulting in cell cycle arrest, but also of drugs that will directly induce apoptosis or slow down the effects of growth factors or directly block cell cycle progression (e.g., Cdk inhibitors);
- They are positioned at the right level of description to represent controlled and uncontrolled cancer proliferation, which is a matter of cell populations, not of a single cell, even if at the single cell level detailed physiological ODE models are available that accurately describe the cell division cycle [17];
- They are deterministic – which may be questionable when small cell numbers are considered, but this is hardly the case when tumors reach a clinical stage, approximately 5×10^5–10^6 cells – and thus make it possible to rigorously predict the behavior of a tissue proliferating freely or submitted to an external therapeutic control. In this respect, theorems may help, not only by telling us for instance that under given hypotheses a considered therapeutic means will not be successful, but also by allowing theoretical comparisons between proposed treatments by their predicted outputs;
- They are amenable to the adjunction of some stochastic components when uncertainty exists on the conservation laws that are represented by the differential equations and conversely when gross behavior must be rigorously predicted – in the mean – from stochastic modeling, such deterministic models can be obtained from probabilistic rules by averaging according to a probability distribution;
- They are computationally much more effective and mathematically (theoretically and numerically) better mastered, allowing the management of larger observation times than the so-called agent-based models that rely on physical and statistical rules applied to the collective behavior of individual cells and are quickly limited to too small cell numbers. It is important to note that comparisons at small cell numbers (roughly less than 10,000 cells, corresponding to a developmental stage at which a tumor is not clinically detectable, but already viable) between the outputs of agent-based (discrete) and PDE (continuous) models, physiologically or spatially structured, are still the object of active research in the community of mathematicians. The aim of such research is not only to obtain so-called 'motifs', experimentally observed, but also to understand the way agent-based models evolve and to give effective analytical laws of their behavior [21], in the spirit, *mutatis mutandis*, of Johannes Kepler for the movements of planets.

As an example, Equation 1 shows a physiologically structured linear PDE model, referred to as the von Förster–McKendrick model, designed to represent the cell cycle in a proliferating cell population [14]. The first equation is a conservation equation, stating that all variations of the population number (or density) in the ith phase are due either to death or to transition to the next phase. The second equation is a boundary condition, stating that, at all times, all cells at age 0 in the ith phase originate from cells that have passed the transition from the previous phase. The third equation is the same as the second, except that it describes cell doubling at mitosis (the Ith phase). The behavior of such a linear model is exponential in time and is shown to be governed, in the mean and for large time periods, by a growth (or Malthus) exponent; a constant times the inverse of the doubling time, which hence may be seen, rather than the total number of cells at time t, as the output of the model, such as when controlled by drugs or by other attempts to re-establish a physiological control. Furthermore, this Malthus exponent may be compared with experimental measurements of cell population growth. Of note, it is an eigenvalue and makes sense only in the case of a linear model, but in the

case of nonlinear models, one may nevertheless calculate eigenvalues in models linearized around particular system states, as seen elsewhere [15,16].

The parameters of such age-structured models, in which age is not a parameter, but a structure variable, not to be identified, can be measured in cultures of cycling cells. This can be performed by fluorescence techniques (fluorescent ubiquitination-based cell cycle indicator [FUCCI] reporters) applied to sets of individual cells, as shown by Sakaue-Sawano *et al.* [22] and using methods such as those presented by Sherer *et al.* [23] to identify transition rates from phase duration probability density functions. Identifying these parameters *in vivo* will certainly be much harder since fluorescent reporters on the cell cycle are not available in living individuals. But at least theoretically, optimized treatments designed from cell cultures can be tested in animal models and then possibly in clinical trials with comparison to reference standard treatments. As previously mentioned, the addition of physiological control from a quiescent phase into the proliferation phase is also possible, as previously described [15,16], but also more classically as described by Mackey [24] with extensions such as those described by Adimy *et al.* [25] and Bernard *et al.* [26].

From a mathematical point of view, the interest of further considering nonlinear models is to obtain richer behavior of the solutions, in particular convergence to a stationary value or sustained oscillations. Physiologically structured PDE models may be transformed into delay differential equation (DDE) models, with supplementary hypotheses and usually with the consideration of a unique phase for the whole cell cycle resulting in one added delay as described by Adimy *et al.* [25], although multiple delays corresponding to cell cycle phases may also be considered, as described by Bernard *et al.* [26]. In particular, this has been carried out for hematopoiesis models, with the additional consideration of a quiescent cell population to the proliferative one and exchanges between them, making them nonlinear by the introduction of a negative feedback function on proliferation. Their application to various hematological diseases is reviewed by Foley and Mackey [27]. DDE models can reproduce the periodic behavior of white blood cell production such as has been observed in cyclic neutropenia or in some forms of chronic myelogenous leukemia.

From a systems physiology point of view, bearing in mind future applications in the clinic, the main purpose of physiologically structured PDE or DDE models is thus to give rigorous conditions for the occurrence of cell population behaviors, such as uncontrolled growth, decay, sustained oscillations or convergence to an equilibrium, that are observed in biological or clinical settings, concerning healthy cell populations as well as cancer growth. The models should be structurally the same in health and in cancer, but with different parameters. It is possible in such models to represent the population dynamics of healthy and cancer cells either independently, when no direct communication exists between the target cells of therapeutic effects and those of adverse drug effects, but also in competing situations, for example by reaction-diffusion equations as described elsewhere [28].

In many occurrences, the shortage of tissue data to identify parameters of these physiological models of tissue growth renders it necessary to deal with more phenomenological ODE models, exponential (valid only at very early stages) [29], logistic, Gompertz or extended from Gompertz (e.g., Hahnfeldt [30]), which have little or no physiological or molecular ground, but are easy to identify on growth curves [31]. Obviously, when such simplified models may be shown to exhibit, for clinical needs, the same behaviors as more complex physiological ones, they will be preferred for the sake of conceptual parsimony (often referred to as Occam's razor [102]) and computational economy. Indeed, mathematical modeling is never a goal in itself: it all depends on the answer to a "modeling, what for?" question when one is concerned with clinical applications. When too few data are available and not much is known of the physiological mechanisms, phenomenological representations such as direct effects on death rates or proliferation rates in ODE models are always an alternative solution, as described by Clairambault [31] or by Panetta *et al.* [32,33].

Yet to theoretically study the behavior of proliferating cell populations in physiological or pathological situations, with external control by drugs on different targets, physiologically structured PDE models of the cell cycle remain the best adapted. The known physiological impact of circadian clocks on cell cycle proliferation has been studied using such theoretical models elsewhere [34]. More generally, physiologically structured PDE models have the ability to include any molecular target for cytotoxic drugs that have different modes of action and are often S-phase or M-phase specific. They are rich enough to potentially account for the molecular action on the growth inhibition of a

tumor tissue of an S-phase-specific cytotoxic drug, such as irinotecan and/or 5-fluorouracil combined with an EGF receptor-targeted antagonist (e.g., cetuximab, presently in use in the clinic [35], or erlotinib [36]) for a corresponding PDE model and many other growth factor-inhibiting drugs.

Mathematical models for physiological molecular PK–PD: molecular, cell, tissue & whole body

In recent years, pharmacological literature has reported many success stories and also less successful attempts towards targeted treatments, such as monoclonal antibodies, associated or not with cytotoxic molecules (e.g., gemtuzumab ozogamicin, recently withdrawn from the US market [103]), new galenic forms or physically aided (e.g., by magnets) vectors for drug delivery as close as possible to tumor tissues. It is not the purpose of this article to review them, but rather to stress the fact that such, sometimes wonderful, targeted therapies cannot do all the jobs, since they may at times show unpredicted toxic side effects that also have to be taken into account and that 'old' drugs, such as 5-fluorouracil, can still be very useful, especially in combined therapies.

Classical anticancer drugs are administered every day in successful combinations into the general circulation at the whole-body level in patients, have toxic side effects on healthy tissues and may be delivered according to differently ordered schedules. The synergistic effects of drug combinations have been well addressed from a rather classical point of view mixing genetics and statistics [37], but not in a physiological perspective. This inevitably comes back by pragmatic clinical considerations to a still present motivation to represent the effects of drug combinations in a physiological and dynamic way by whole-body PK–PD models.

Indeed, relating blood PK to therapeutic efficacy and toxicity in a direct way is hopeless if one does not take into account not only blood PK, but also tissue PK, together with tissue PD models – or better said, 'PD-population dynamics' models of the effects of drugs on both wanted and unwanted targets in cell populations, tumor and healthy. Various whole-body compartmental PK ODE models have been published in recent years in excellent journals dedicated to PK and its clinical applications in oncology. They represent state-of-the-art pharmacological modeling, sometimes being 'semiphysiological' [38]. Rather than reporting these compartmental models that are easily extendable to user-friendly software for population studies, this article will focus on physiological PK–PD modeling at the level of an individual patient, which in my opinion is the immediate future of modeling for therapeutics.

As mentioned in the first section, drugs act at the molecular level in peripheral tissues but are delivered (possibly after previous intestinal absorption) into the central blood compartment at the whole-organism level. Physiological representations of the fate of drugs must take into account this multiscale setting and rely on equations dealing with drug concentrations in blood and tissues for whole-body physiologically based PK (WBPBPK, a term coined by Malcolm Rowland [12]) and molecular reactions based on law of mass action or Michaelian kinetics for intracellular PK and PD (hence, WBPBPKPD). Physiologically based modeling implies going beyond roughly descriptive blood PK parameters (e.g., half-lives, maximum concentration, peak concentration time and area under curve) and as much as possible involves tissue characteristics, such as enzymatic activities for cell PK and in particular DNA double-strand breaks for cell PD.

In more detail, PK–PD molecular model design for anticancer drug optimization is concerned with drug concentrations in the plasma, in organs and in cell populations that are drug targets, be they desired (tumor tissues) or not (healthy tissues subject to toxic side effects). The molecular choice of mechanism description, both for PK (drug transformations from its input in the general circulation) and for PD (action on the target at the cell and tissue levels) makes such models amenable to take into account by different parameters genetic (e.g., enzymatic polymorphism) and epigenetic variations between individuals. This may subsequently lead to clearly identifiable (provided of course that corresponding biomarkers are available, which unfortunately is not always the case) physiological characterization of different profiles directed towards individualized treatments. To date, most efforts have been made on drug processing enzyme genetics, with limited success [6], but it is not unlikely that by taking physiological differences between patients, which are not always explained by genetics, into account in drug processing, one can improve this situation. This is at least one non-negligible motivation to study PK–PD on physiological rather than only genetic bases.

Numerous PK–PD models based on differential equations exist for various anticancer drugs and some of them are molecular based, but not all of them are physiologically based (they more often rely on compartment design that is phenomenologically guided by drug

blood concentration curve fitting, with hardly any physiological considerations) and even fewer among them are whole-body designed.

An important issue for these models is the experimental identification of their parameters, since they include much more than easily accessible blood PK characteristics. Dealing with tissue PK and PD, they must be identified initially in cell cultures (to begin with, immortalized cell cultures, then *ex vivo* transplants, which are shorter lived). Then, passing from *in vitro* to *in vivo*, in laboratory animals by using healthy tissue samples and samples from xenografts standing for *in vivo* host tumors and eventually in clinical trials, by using inverse problem analysis techniques, for example. As a whole this is a long process and hardly ever yields effective quantitative predictive models in a reasonable timeframe. More modestly, qualitative predictions may be obtained to compare different treatment schedules between them and then test these predictions in clinical settings, with the outcomes being response to treatment and long-term survival.

An example of a model for WBPBPK for capecitabine has been reported [39]. A theoretical proposal of an essentially intracellular PK–PD model, nonetheless including whole-body infusion, for the combined delivery of 5-fluororacil with folinic acid (a potentializing association used in the treatment of colorectal cancer) has also been described [10]. An illustration of the output of this ODE model with periodic drug infusion is shown in Figure 3. The equations of the ODE system rely on the law of mass action and Michaelian kinetics when enzymes or transporters are concerned according to what is known of the intracellular physiology of drug processing. Other models based on the same physiological multiscale principles, with law of mass action and Michaelian kinetics, in particular for irinotecan [Ballesta A, Dulong S, Abbara C *et al.*: A combined experimental and mathematical approach for molecular-based optimization of irinotecan circadian delivery. Manuscript Submitted] [40] and cytosine arabinoside [104], are currently being designed and experimentally identified in cell cultures or in laboratory animal models. The last of these two models is in fact a hybrid model, where continuous differential equations for drugs and for regulatory proteins exert their actions on a discrete agent-based model for cell population dynamics [104].

If one takes into consideration within-subject variability in the response to drugs, as discussed in the first section of this article, the impact of circadian clocks on drug processing should be represented by periodic functions to modulate the maximal activity (V_{max}) of enzymes or transporter proteins. Such periodic functions may be plain sine waves in the most elementary form of circadian modeling, but also much more detailed ODE models of the clock [41], when detailed knowledge of their function must be taken into account. This may be the case when a reverse impact of cytokines on the clock is to be considered, such as tumor-emitted cytokines, which has already been shown experimentally [42] and clinically [43]. In this case, targets for toxicity to the central and to the peripheral clocks may also be added to the healthy tissue toxicity part of the whole-body model, since some anticancer drugs have been proven to induce circadian clock disruption [10].

In the same way, in a whole-body perspective going beyond the representation of only proliferating tissues (healthy and tumor), targets of toxicity for nonproliferative cell populations, such as cardiac (anthracyclines) or neurological (e.g., oxaliplatin) should be considered. Beneficial effects on the surrounding tissues of additional drugs, such as anti-inflammatory, antiangiogenic, matrix metalloprotease inhibitors and pH-modifying immunostimulators may also be represented according to their effective associations in specific combined treatments used in the clinic. They are numerous and it is not the purpose of this article to review them, assuming that cancer is primarily a disease of uncontrolled cell and tissue proliferation. When molecular models are available to take these effects into account, they should be used, depending on the treatments that are under study in the clinic.

Therapeutic optimization: optimization algorithms for therapeutic control of tissue proliferation

The practical aim of these models, representing both the system to be controlled on the one hand (proliferating cell populations in a whole-body setting) and the therapeutic control methods on the other hand (drugs, their fates and their effects on proliferating tissues in a living organism), is to give clinicians a rationale to handle treatments of cancer in the best possible way. By 'best possible way' it is meant that treatments of cancers are always a compromise between therapeutic efficacy, that is destruction of tumor cells (the objective) and preservation of physiological functions by healthy cells (the constraint).

An optimization problem under constraints is the mathematical formulation of a trade-off: given an objective and a constraint, its solution, when it exists, is a control function on the system

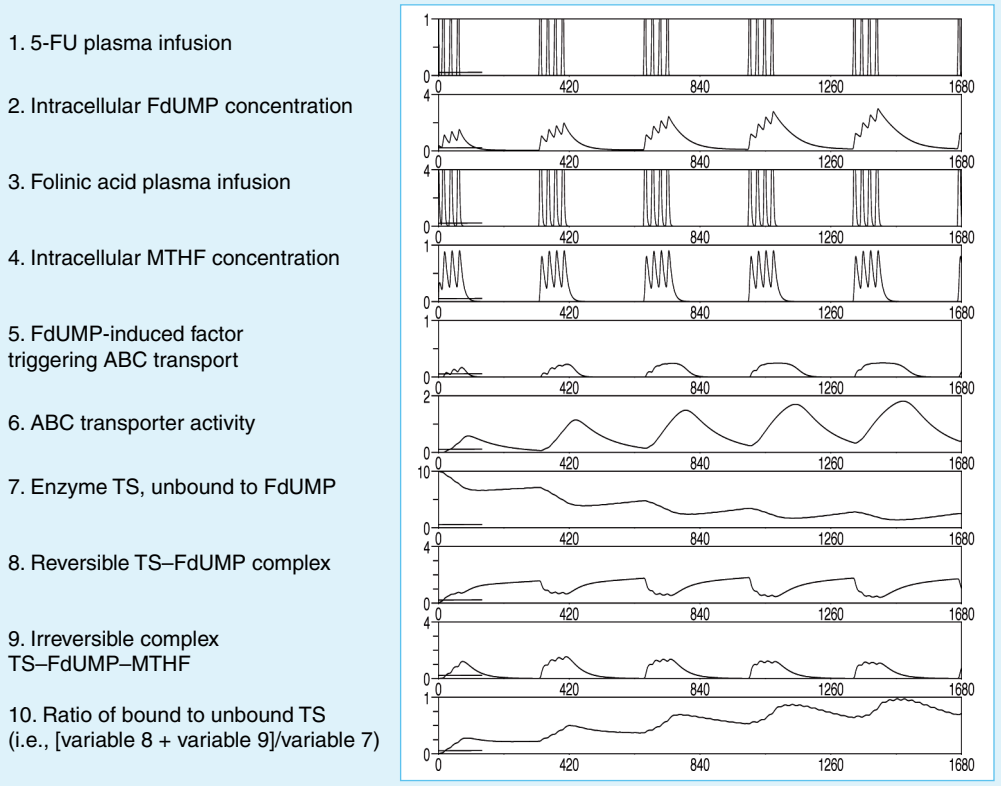

Figure 3. Simulation of an ordinary differential equation system representing intracellular concentrations and effects on the target enzyme thymidylate synthase of a combined treatment by 5-fluorouracil and folinic acid. A total of nine variables constitute this system, from periodic plasma infusion of the two drugs until the decay of free TS due to reversible and then irreversible binding to FdUMP. 5-FU is transformed in the cell to its active form FdUMP and similarly folinic acid is transformed to MTHF. An ABC transporter, expelling the drug from the intracellular medium and triggered through an assumed intermediate factor by FdUMP, is here supposed to be responsible for drug-induced resistance to the treatment. FdUMP binds to TS and the secondary binding to them of MTHF makes the complex indissociable, resulting in free (unbound) TS loss. The last track is the ratio between TS–FdUMP bound complexes, reversible or not, and free (unbound) TS. Times are in hours, vertical units are arbitrary. Further details can be found elsewhere [10].
5-FU: 5-fluorouracil; ABC: ATP-binding cassette; FdUMP: Fluorodeoxyuridine monophosphate; MTHF: Methylene tetrahydrofolate; TS: Thymidylate synthase.

(a treatment, that is a drug delivery schedule in the case of therapeutics) that will maximize the objective function while satisfying the constraint. Most treatments of cancers that use cytotoxic or cytostatic drugs arrest or slow down the cell cycle in fast-renewing cell populations and this holds true not only for tumors, but also for fast-renewing healthy tissues, such as gut, skin or bone marrow. Efficient anticancer therapeutics manage to hit cancer cells without damaging healthy cells too much, but rules to achieve this goal are rare.

One way to do it is to take advantage of differences between healthy and cancer cell populations with respect to observed circadian rhythms of drug toxicity.

Using a simple cellular automata model of the cell division cycle in cell populations [44,45], it has been suggested that desynchronization between phases of the division cycle, that is an extended overlap of phase transition probabilities between cells in the cell population (assumed to be the case for cancer cells) together with lesser sensitivity to gating at checkpoints by circadian clock controls, results in enhanced sensitivity to drug damage. This is true provided that cell cycle phase-sensitive drugs, such as 5-fluorouracil, are delivered according to a circadian scheme applied at times when healthy cell populations (assumed to be better synchronized and better entrained by circadian gating) are relatively protected from drugs.

Coming to somehow support from a theoretical point of view a current hypothesis according to which cancer cell populations are less synchronized with respect to cell cycle timing, preliminary unpublished mathematical results, that is, theorems, not simulations nor experiments, using physiologically structured PDE models, show that desynchronization between cells yields

faster growth (i.e., results in higher Malthus exponents). This idea is currently being experimentally investigated by cell cycle fluorescent reporters (e.g., fluorescent ubiquitination-based cell cycle indicator reporters) [22] recorded in samples from healthy and tumor tissues. A conjecture is first that such desynchronization might be a hallmark of cancer and second that lesser sensitivity to synchronizing signaling from the hypothalamic circadian clock might be responsible for this loss of synchrony, a marked difference between healthy and cancer tissues that could subsequently be exploited in therapeutics. This conjecture is currently investigated, among other questions, in the European Systems Biology Research Network (ERASysBio) 'C5Sys' [105].

Using a completely different, deterministic model of the PK–PD of a noncell cycle phase-specific drug (e.g., oxaliplatin) representing simultaneous growth of healthy and cancer tissues by simplified ODEs, with the (rather strong, but not without experimental support) assumption of 12-h-shifted sensitivity peak time to the drug in the two tissues, it has been shown that a numerical optimization technique, as developed in an earlier work [46], yielded a proposed optimal drug delivery time schedule, maximizing antitumor efficacy under the constraint of absolutely keeping unwanted toxicity on healthy cells (in that case villi cells in the gut) under a tolerability threshold. This is illustrated in Figure 4.

This was a proof of concept that optimization of therapeutic control under dynamic toxicity constraints is possible. Others have also tackled this problem in more classical settings, proposing theoretical solutions in both cell-cycle-specific and nonspecific cases [47].

Yet, complex as it may seem to manage simultaneously healthy and cancer populations toward this goal, another even more difficult issue is encountered in the clinic when empirically optimizing anticancer treatments; the need to avoid the emergence of resistance to drugs in cancer cell populations as much as possible. The mechanisms of drug resistance are many and not all are understood. Some are due to unpredictable mutations, others seem to be drug induced, that is triggered by long exposure to the same drug (rather than high doses: on the contrary, giving high doses in a short time could be beneficial to patients, as long as no heavy toxic side effect occurs [48]). Some may be due to intracellular enhancement of physiological mechanisms such as overexpression of ATP-binding cassette transporters or drug detoxication enzymes and others may be due to resistant cell selection by mutations in the population of (genomically unstable) cancer cells under the pressure of drugs, according to a 'cell Darwinism' hypothesis. It is not clear whether acquired drug resistance in cancer cells is reversible or not. Tackling the problem of the emergence of drug resistance in cancer cells is thus much more difficult than optimizing under an unwanted toxicity constraint, and to my knowledge, no satisfactory representation has been proposed for it so far. It is nevertheless clear that, again, it will be necessary to consider the level of proliferating cell populations in order to make advances in the right direction. If pathophysiological mechanisms are unraveled and may be represented by molecular-based equations, then optimization algorithms associating several anticancer drugs, to limit the emergence of resistance to one if it were given in monotherapy,

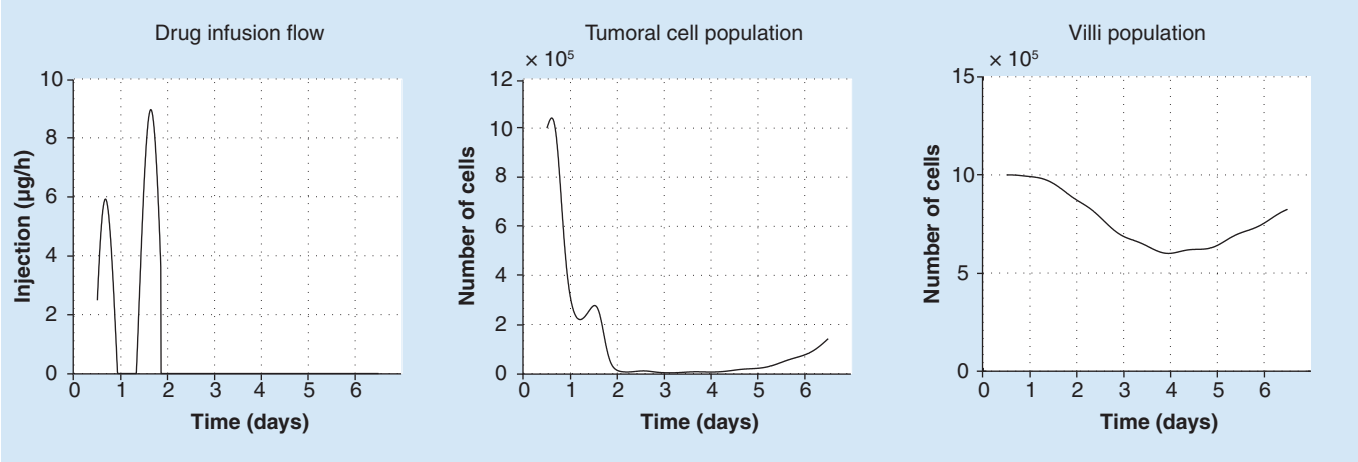

Figure 4. Optimized drug infusion flow over 2 days followed a recovery period in a 1-week chronotherapy course intended to limit (without eradication) cancer growth while maintaining toxicity to healthy villi cells under an absolute tolerability threshold, here the preservation of at least 60% of the equilibrium population cells. This 1-week course must be followed by subsequent courses to prevent tumor regrowth. Further details can be found elsewhere [17].

may be used together with limited toxicity constraints, as has been detailed from a statistical viewpoint [37]. But much remains to be done both experimentally and theoretically to better understand the mechanisms of drug resistance in cancer cell populations, in particular by adaptive dynamics in a genetic evolutionary perspective, if we want to take into account the limitation of emergence of drug resistance as a constraint in an optimization problem. To date, optimization has mainly been performed on ODE models, but a genetic evolutionary perspective taking into account cell selection pressure by drugs, such as in long exposure schemes, as an environmental factor should again drive us back to PDE models, with a genetic trait occurring as a structure variable [49–51].

It should also be noted that such models, designed to improve therapeutic control on tissue proliferation, are open-loop. That is, they do not claim to achieve optimized control using information from the effect of the treatment on the target system, as do automated closed-loop systems designed for the real-time control of glycerin or blood oxygenation. As previously mentioned, the feedback in oncology comes from the clinic and the complexity of the treatment response (therapeutic response in terms of radiologic image shrinkage; various toxicities, clinically detectable or only biologically; emergence of drug resistance; positive or adverse effects on the immune system) makes it hardly amenable to automatic correction in an optimal control perspective, as in the case of the trajectory of an airplane with a given destination and rather simple constraints, for example.

Finally, it should be noted that theoretical optimization of anticancer treatments as discussed here is not limited by number of drugs used in combination therapies, making it a potentially useful tool to mimic and rationalize modern treatments of cancer, which in their vast majority rely on the associations of drugs acting on different cell targets.

Individualizing treatments in oncology: the present & the near future

From the point of view of personalized medicine, the models sketched above for tissue proliferation (and its control) and for drug disposition present the advantage to be physiologically adaptable to individual patients insofar as they depend upon parameters that are in principle (or will be some day) identifiable by biological samples. Parameters of enzymatic activity that are the affinity constant (K_m) and maximum velocity of reaction (V_{max}) in a Michaelian representation (mean, peak and trough V_{max} if one assumes the fact that V_{max} may be subject to circadian variations) may be considered as characteristic of each individual for drug processing enzymes. When these enzymes present genetic polymorphism with a clearly identified gene, then one can hope that reading any patient's complete genetic code on a cellular phone, which already belongs to a very close future given present fast developments of information and communication technology [106], may give an insight to her or his biological characteristics, making it possible to adapt treatments to her or his case. Of course, this is not specific of cancer treatments, but given that the genomic instability is one of the hallmarks of cancer, finding genetic mutations responsible for mutated forms of proteins, such as KRAS for the possible treatment of metastatic colorectal cancer by EGF receptor antagonists [52], may prove quite valuable. Of note, pharmacogenomic profiling in view of treating cancer by targeted therapies seems to have been more successful recently when searching for mutations or chimeric fusions of genes coding for proteins involved in pathological intracellular pathways (e.g., *KRAS*, *BCR-ABL*, *PML-RARA*, *Flt-3*, *Her2*) than of specific drug-processing enzymes and more should come from genome-wide association studies. However, this situation may be due to the fact that the level of gene description, rather than the protein activity level, has been the most explored in the recent past.

Indeed, proteonic activity is not always dependent on only one gene and even assuming that all genes coding for a protein have been identified, epigenetic modifications may also explain the silencing of tumor suppressors or overexpression of oncogenes associated with cancer [53]. Examples of epigenetic variations that might explain fitness of cancer cells to a changing environment have been discussed [54]. Together with the fact that modifications of the intracellular or extracellular environment (in particular perturbed synchronizing messages from a disrupted circadian clock) may induce enhancement of tumor progression [55], these observations suggest that genetic tests, valuable though they are, may not be enough to catch between-subject and within-subject variability in cancer progression and in patients' responses to drugs. Hence, modeling at a physiological level (proteins and protein activity) of both tissue proliferation and drug fate in organisms may be

a preferable way to work toward the prediction of response to anticancer treatment and its optimization. Of course, many biomarkers remain to be designed to make physiologically based mathematical models identifiable from biological samples and clinically applicable, but this is a general issue that cancer biology has to cope with in the immediate future.

Conclusion & future perspective

Apart from what is discussed in this article on the issue of designing more biomarkers to adapt therapies in individual patients, or rather in groups of patients (e.g., slow and fast acetylators), according to phenotypic – rather than only genotypic – profiles, other tracks remain to be explored. Areas where progresses in experimental and theoretical biology (including mathematical modeling) are expected to develop include:

- Immunotherapies: that is using the patient's individual built-in defenses against tumor cells;
- Better understanding of the emergence of resistance in cancer cell populations: what are the concerned mechanisms that may differ from one subject to the other, from one situation to the other and how should they be overcome by adapted combined therapeutic strategies? (See previous section regarding optimization);
- Noncell-killing therapeutics, with the objective to be less toxic to healthy tissues: instead of only killing tumor cells, such therapeutics could let them stagnate and then naturally disappear in an environment in which they cannot thrive, to the advantage of healthy cells. This is at least the case for the treatment of a rare form of leukemia, acute promyelocytic leukemia, in which the delivery of a redifferentiating agent (combined or not with a classical cytotoxic drug) allows the differentiation blockade to be removed and cures up to 80% of patients [56];
- Systems biology representations toward the prediction and optimization of therapeutic outcome: not in an exhaustively descriptive way of intracellular signaling networks but for specific diseases and their instances in individualized patients, in particular by physiologically based mathematical modeling, so as to identify so-called 'hubs' in intracellular signaling pathways and hit them by targeted therapies [57]. This is indeed the case of the example of acute promyelocytic leukemia, where the protein responsible for the differentiation blockade is the chimeric protein encoded by the *PML–RARA* fusion oncogene, which is successfully hit by all-*trans*-retinoic acid and other mutations, such as Flt-3 duplication [56], and is already the object of therapeutic assays in the clinic.

Systems biology applied to drug discovery, disposal and delivery optimization has already been called systems pharmacology [58]. It usually constitutes the description of physiological networks, that is intertwined intracellular, tissue-level and whole-body signaling pathways, representing the fate of drugs from their infusion into the general circulation until their effects are recorded at the molecular and whole-organism scale. These signaling pathways are described by ordinary differential equations; the parameters and control functions in these equations contain both between-subject and within-subject variability, which makes systems biology and systems pharmacology models amenable to individualization. In the case of cancer treatments, I propose the addition to this perspective of a representation, in terms of physiologically structured population dynamics under control, of the evolution of the cell populations that are targets of the drugs (wanted or unwanted) in order to predict treatment outcomes on the actual drug targets and not indirect indexes. At the other end of the pharmacotherapeutic chain, I propose a systematic use of optimized therapeutic control algorithms under the constraints of limiting adverse drug response effects, which is already rather well understood and quite reachable, and also emergence of drug resistance in cancer tissues, which is a more distant objective due to the complexity of the involved mechanisms. Both of these prospects call for enhanced collaboration between mathematicians, biologists, pharmacologists and clinicians, which could be better obtained in integrated cancer research centers, some of which already exist, thus far mainly in the USA.

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Executive summary

Pharmacology of cancer, variability in drug response & personalized medicine

- Between-subject and within-subject (circadian variations) variability in the response to drugs is a constant in personalized medicine and they should be considered to improve treatments, especially in the case of cancer, where therapeutic progress is almost at a standstill.
- Systems biology is called upon in this context to represent, by physiological rules and equations, both the means of action (the fate of drugs in the organism) and their targets, that is cell populations, both healthy and tumoral.

Mathematical models of cancer & its pharmacological treatments: a systems biology viewpoint

- Mathematical models and methods should be developed along three axes:
  - Representation of cell proliferation by physiologically based models of the cell cycle in cell populations.
  - Pharmacokinetics–pharmacodynamics (PK–PD) of anticancer drugs.
  - Optimization algorithms to optimize multidrug treatments delivered to a central blood compartment.

Mathematical models of cell proliferation & its control

- Physiologically structured partial differential equation models are the best models to theoretically study the effects of anticancer drugs on proliferation in cell populations.
- They can be complexified or simplified according to the problem under study and the availability of data to identify their parameters.

Mathematical models for physiological molecular PK–PD: molecular, cell, tissue & whole body

- PK–PD of anticancer drugs should be represented by ordinary differential equation models in a multiscale setting: whole-body, tissue, cell and molecular effects.
- Parameters of these models characterize genetic polymorphisms and other between-subject variations.
- Their modulation by inputs from circadian clocks must not be neglected.

Therapeutic optimization: optimization algorithms for therapeutic control of tissue proliferation

- Optimization algorithms yield solutions, in terms of best proposed drug treatments (e.g., delivery schedules to be implemented in programmable pumps in the clinic) to an optimization problem, that is maximizing tumor cell kill under the constraint of limiting unwanted adverse drug effects and if possible (but more difficult) also limiting the emergence of resistant tumor cell subpopulations.
- They are not limited in number of drugs and may yield optimized combined therapies of different drugs.
- They take into account individual physiological characteristics and yield optimal individualized therapies.

Individualizing treatments in oncology: the present & the near future

- Individualization of treatments with a systems biology and systems pharmacology viewpoint must certainly take into account pharmacogenomic studies, especially in the near future when anyone may carry their genome sequence in their pocket.
- Having the proteome level and the epigenetic level as a goal as well as cell and tissue environmental modifications that may be specific to a given individual is manageable in a systems biology perspective, provided that one has good biomarkers (which is still an unsolved issue in individualized medicine in general).

Conclusion & future perspective

- New frontiers should include immunotherapy, theoretical and experimental advances in the understanding of drug resistance and noncell-killing therapeutics, together with a search for high-interest targets for therapies, such as the BCR-ABL chimeric protein for the treatment of chronic myelogenous leukemia.
- To the usual setting of systems biology that investigates intracellular networks of signaling pathways should be added, in the case of cancer (a disease of proliferation), a cell population dynamics perspective describing the cell cycle in cell populations, both healthy and tumoral.
- Optimization of anticancer treatments must be the ultimate goal in modeling cancer growth and therapy; a physiological multiscale systems biology perspective should be a constant concern toward this goal, as it potentially holds all ingredients to personalize treatments when biomarkers are available.

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