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Physiologically Based Mathematical Models to Optimize Therapies Against Metastatic Colorectal Cancer: A Mini-Review

Annabelle Ballesta[1,2*] and Jean Clairambault[1,2,*]

[1]*INRIA BANG project team, INRIA Paris-Rocquencourt, BP105 78153 Le Chesnay, France.* [2]*UPMC Laboratoire Jacques-Louis Lions, UMR 7598, Boite courrier 187, 75252 Paris, Cedex 05, France*

Abstract: Understanding and improving the effects of combined drug treatments in metastatic colorectal Cancer (mCRC) is a multidisciplinary and multiscale problem, that can benefit from a systems biology approach. Although a quite limited number of active drugs have been approved for clinical applications, a variety of combined delivery regimen options are actually used in the clinic, so that choosing between them, or designing new ones, is not an obvious task, which calls for some rationalization based on physiological principles. We propose some physiologically based molecular pharmacokinetics-pharmacodynamics models for the main cytotoxic drugs used in the clinic and call for others describing more recently used agents, such as associated with monoclonal antibodies. We also advocate simultaneously designing models of the proliferating cell populations under therapeutic control, as cancer is primarily a disruption of physiological control on tissue proliferation. These two types of models are based on differential equations to continuously describe both the fate of drugs in the organism, from infusion until pharmacological effects, and their impact on the proliferation of cell populations, healthy and tumor. The multiscale nature of colorectal cancer, from the disruption of intracellular pathways to tumor growth observed at the macroscopic level, together with its frequent multilocal extension by simultaneous metastases in various healthy tissues of the organism at the time of diagnosis, and later, call for multiscale mathematical models. We thus propose a multi-level vision of cytotoxic drug use in the clinic, in which the weapon in the hands of clinicians, a drug combination regimen, the targets -wanted and unwanted –on which it exerts its effects, molecular pathways in proliferating cell populations, and the environment of the latter in a whole organism, are all considered in order to design a rationale for appropriate shooting, i.e., treatment optimization under patient-tailored constraints.

Keywords: Colorectal cancer, mathematical modeling, anticancer therapy optimization, personalized medicine.

1. INTRODUCTION

1.1. Basic Facts on Colorectal Cancer

Colorectal cancer was in 2008, far behind lung cancer, the second most frequent cause of mortality by cancer, both sexes merged, in Europe (12%) and in the Americas (8%), according to the International Agency for Research on Cancer of the WHO (source: http: //globocan.iarc.fr). Survival rates for patients with colorectal cancer are 83.2%, 64.3% and 57.6%, respectively 1, 5 and 10 years after diagnosis [1]. 5-year survival rate is 90.1% when the disease is discovered at a localized stage whereas it drops to 69.2% when metastases in adjacent organs are detected and to 11.7% when the cancer has spread to distant organs [1]. Therefore, improving therapies against metastatic colorectal cancer (mCRC) remains nowadays a clinical challenge.

It is generally estimated that about 95% of colorectal cancers are adenocarcinomas, i.e., tumors resulting from uncontrolled growth of the glandular tissue of colorectal mucosa. Metastases are frequent, hit preferentially the liver, but also frequently the lung and the peritoneum. They may reveal the cancer, which is then at a stage with bad prognosis, requiring extended courses of chemotherapy, prior to any surgical resection when these metastases are numerous or adherent to vital parts of organs [1]. Therefore, physiologically based mathematical models designed to optimize treatments against mCRC may take into account the disseminated character of cancer cell populations, which are often located in a healthy cell environment that will also be exposed to administered anticancer drugs.

1.2. A Whole-body Multi-scale Disease

Cancer in general and mCRC in particular may be primarily characterized by a loss of physiological control on cell proliferation and survival, and secondarily by a propensity of tumor cells to invade surrounding tissues [2]. Drugs addressing the invasive potential of tumor cells (inhibitors of extracellular matrix digesting proteinases) having thus far proved disappointing [3], it is mainly on the replicative potential of tumor cells that act the few drugs that have been recognized as active on mCRC. These drugs act by slowing down or arresting the cell division cycle, which is the universal process by which a cell, healthy or cancer, becomes two. This cell cycle arrest may then lead to cell apoptosis [4].

It is thus appealing to search for intracellular signaling pathways that are essential for the cell proliferation and survival, and more precisely for key steps in these pathways so-called 'druggable targets'. Nevertheless, such pathways are likely to be modified in cancer cells which are characterized by a high ability to adapt to unfavorable environments. As a consequence, drug activity may be different in normal tissues compared to tumor ones which may lead to treatment-limiting side effects at the level of the whole organism. The search for druggable targets, interesting as it may seem, must thus be considered by the effects on the different cell populations that are actually hit by the drugs. Indeed, simultaneous study of the patient's tumor and healthy tissues may allow the identification of determinant mutations or molecular differences which could then be exploited in the optimization of healthy and cancer cells specific response to treatments.

However, intracellular mathematical models need to be linked to tissue level models in order to measure therapeutic effects on proliferation, as proliferation can only be considered at the level of a cell population. Hence the natural idea to consider models of the cell division cycle in proliferating cell populations, healthy and cancer. A physiological representation of the cell cycle, with explicit mention of cell cycle phases (G0-G1/S/G2/M), may be required if the drugs of interest are phase-specific. Finally, therapeutic optimization may imply to take into account efficacy together with all induced toxicities at the level of the organism. This may be performed by the use of whole-body physiologically based mathematical models in which compartments represent the simulated organs.

*Address correspondence to this author at the UPMC Laboratoire Jacques-Louis Lions, UMR 7598, Boite courrier 187, 75252 Paris Cedex 05, France; Tel: +33 1 44 27 91 70; Fax: +33 1 44 27 72 00;
E-mails: annabelle.ballesta@inria.fr; ean.clairambault@inria.fr

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The liver is often modeled for its major part in drug metabolism, as well as the healthy cell populations which constitute the main toxicity targets, and the tumor, to assess drug efficacy.

1.3. Approved Chemotherapy Regimens Against mCRC

Nowadays, three cytotoxic drugs have been approved by the US Food and Drug Association (FDA) for chemotherapy against mCRC: 5-Fluorouracil (5-FU), oxaliplatin (l-OHP), and irinotecan (CPT11). 5-FU has been administered to mCRC patients since 1957 and still remains the main component of current therapeutic strategies. For long, 5-FU monotherapy was the sole effective treatment against mCRC, increasing the median overall patient's survival to approximately 12 months, compared with 4-6 months with palliative care [5]. Its association with folinic acid, also known as leucovorin (LV) amplifies 5-FU activity which results in increased response rates and patient overall survival [5]. Moreover, prolonged continuous exposure to 5-FU, which was made possible by indwelling central venous catheters and the use of infusion devices achieved better response rates and lower toxicities compared with daily bolus [6]. The oral prodrug of 5-FU, capecitabine, was approved by the FDA for the treatment of mCRC in 1998. It is converted to its active metabolite by three different enzymes, including thymidylate phosphorylase which tends to be more expressed in tumors than in normal tissues. Thus, capecitabine may induce lower toxic effects than 5-FU bolus [7]. However, the advantage of oral fluoropyridimines compared with 5-FU infusion is still under study [8].

In the early 2000s, 5-FU associated to LV was combined to newly-approved anticancer drugs: oxaliplatin (FOLFOX regimen) or CPT11 (FOLFIRI regimen). Exposure to both FOLFOX and FOLFIRI, irrespective of their sequence, further increased patients overall survival. Indeed, survival rates have risen from 35% at 5 years in the 1980-1994 period to 57% in 2001 [7]. The regimen combining all three drugs (FOLFOXIRI) achieved superior response rate and survival compared with FOLFIRI but induced severe toxicities which advocate for patient selection [5]. Meta-analysis of clinical trials do not conclude firmly on any global guidance concerning optimal chemotherapy cycle durations and optimal drug infusion schemes, which opens the way for personalized medicine [5, 8, 9].

The three above-mentioned cytotoxic drugs may be combined to targeted monoclonal antibody therapies in the treatment of mCRC. Cetuximab is a monoclonal antibody (mAb) against Epidermal Growth Factor Receptor (EGFR) which binds to the extracellular receptor and induces its internalization thus reducing cell proliferation rate [10, 11]. Cetuximab use in combination with either FOLFOX or FOLFIRI is advised in patients who are Kirsten rat sarcoma gene (KRAS) wild-type. Indeed, two independent randomized clinical trials demonstrated a significant improvement in response rate and progression-free survival with cetuximab-FOLFIRI or cetuximab-FOLFOX compared with FOLFIRI or FOLFOX alone [12, 13]. Panitumumab is a human immunoglobulinG2 mAb against EGFR with proven clinical activity in KRAS wild-type metastatic colorectal carcinoma. Its combination with chemotherapy regimen is being further evaluated in phase III trials [7, 14].

Bevacizumab is a mAb which targets Vascular Endothelial Growth Factor (VEGF), one of the most important pro-angiogenic proteins [15]. Several clinical trials have demonstrated that its combination with cytotoxic drug regimens may increase patient survival compared with the sole administration of standard chemotherapy. More recently, the FDA approved the angiogenesis inhibitor ziv-aflibercept for use in combination with FOLFIRI regimen to treat adults with mCRC whose tumors are resistant to oxaliplatin-containing chemotherapy regimen.

A current clinical challenge lays in the resistance to chemotherapy against mCRC which may appear over time due to drug-induced adaptation of cancer cells [7]. Molecular mechanisms of chemoresistance can involve drug uptake when influx transporter amount is decreased, or drug export when cancer cells increase the activity level of their efflux pumps (ATP-Binding Cassette transporters). Expression of enzymes responsible for drug metabolization/activation or inactivation can also be decreased or enhanced to allow cell resistance. Resistance mechanisms can also involve a decrease in molecular target levels. Finally, cancer cell may enhance DNA repair mechanisms or inhibit the apoptotic machinery by overexpressing anti-apoptotic proteins or repressing expression of pro-apoptotic ones [7].

2. MATHEMATICAL MODELS: PROLIFERATION OF CELL POPULATIONS AND FATE OF DRUGS IN THE ORGANISM

2.1. Mechanistic VS. Phenomenological Mathematical Modeling

Representing proliferation in cell populations may be done using a global model of the general form $dN/dt = \{\text{birth}(N) - \text{death}(N)\}.N$, where 'birth minus death' is the proliferation rate of the population of size N, as proposed in simple population dynamics models (e.g., [16–18] and many others). But it is then impossible in such a global (phenomenological) setting to have access to different molecular mechanisms of control that can be exerted on apoptosis, on cell cycle transition blockade (in particular as performed by the protein p53 and cyclin dependent kinase inhibitors), or on slowing down the cell cycle in the G1 -phase by cytostatic drugs for instance. In the same way, to try and understand what the synergies between drugs in anticancer treatments are, and how they can be optimized, one can take advantage of molecular representations of the modes of action of the drugs, when they are known. In this sense, a model will also be said to be physiological (or mechanistic) by opposition to a non molecular one, in which coarser statistical characterizations by linear and bilinear (in the case of interactions) effects will be used. This goes also for population models of PK-PD, in which characterizations of drug responses are based on statistical measurements of global drug effects in populations of patients (in particular for drug associations), without taking any account of molecular considerations.

We advocate here (and elsewhere, see e.g., [19, 20]) the use of molecular representations of the action of drugs as physiological bases for treatment optimization (Fig. **1**). Drugs with different molecular targets can thus be studied as outputs of chains of chemical reactions, and their delivery regimens in combinations at the whole organism level can be varied and optimized. Indeed, current clinical chemotherapy against mCRC involves combinations of several drugs which have different mechanisms of action on intracellular targets in proliferating cell populations, all of which impact the division cycle. By representing the effects of drugs on these physiological targets, with their consequences on proliferation of the cell population, it is theoretically possible, by tuning their delivery in time, to obtain the best possible therapeutic results and thus to design optimized drug infusion schemes to be implemented into programmable devices in the clinic.

Furthermore, such physiological representations by molecular PK-PD and cell population dynamic models clearly involve identification of parameters (such as enzyme kinetics of drug metabolism) of these dynamical systems to allow for predictions of therapeutic outputs. This is a non trivial problem, which often can be solved only for simplified forms of the models at stake, by making use of appropriate biomarkers, not easily found. But when it is solved, such parameters will give access to physiological characterization of the drug response mechanisms in a given patient and will then allow for anticancer therapy personalization on physio-

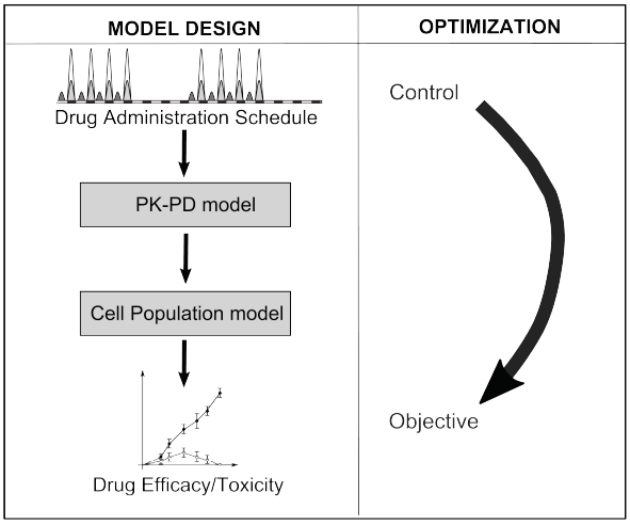

Fig. (1). Theoretical framework to optimize drug administration.

logical, rather than phenomenological and statistical, grounds. Being thus able to 'profile' a patient from physiologically relevant characteristics at the level of his/her organism, involving cell population pharmacodynamics as well as blood and tissue PK, population PK-PD can then be developed in populations of patients on the basis of such physiological characteristics, that go beyond pharmacogenomic typing, as exposed in a recent review article [21].

2.2. Models of Intracellular Pathways Involved in Cell Proliferation and Survival

In order to optimize cell response to treatment it is first necessary to accurately model the intracellular mechanisms that occur in the absence of drugs, drug effect being added as a second step. Intracellular pathways of interest consist in the ones targeted by the considered drugs. In the case of anticancer drugs approved against mCRC, one should focus on gene networks involved in cell proliferation and survival.

First, the p53 protein is known to have a central role in cell response to cytotoxic insults. When DNA damage is detected, p53 blocks the cell cycle, triggers DNA repair mechanisms and/or launches apoptosis. Simplest mathematical models of p53 regulation represent the negative feedback that the protein Mdm2 exerts on p53 expression [22, 23]. Another class of models takes into account the spatial organization of the cell and shows that oscillations in the p53-Mdm2 network can be achieved by considering the location of biological processes [24-26]. The same authors further detailed spatial components including both a nuclear membrane and the structure of cytosolic microtubules which resulted in even more robust oscillators [25, 27].

The mammalian cell cycle is the phenomenon by which a cell becomes 2. It is composed of 4 successive phases: Gap 1 (G1), Synthesis (S), Gap 2 (G2) and Mitosis (M). It results from underlying intracellular gene networks organized in negative and positive feedback loops. Sequential activation of different cyclin/Cdk complexes controls the successive phases of the cell cycle. Several theoretical models of portions or entire mammalian cell cycle have been proposed and are all based on nonlinear ordinary differential equations (ODEs) [28-31]. They intend to better understand the structure of the gene network and its response to gene knock-outs and drug-induced perturbations.

Finally, several published works propose mathematical modeling of apoptosis that is the phenomena by which a cell triggers its own death. Some of them model all pathways to apoptosis from the apoptotic stimulus to the actual cell death [32–34]. Others focus on the final part that is the caspase cascade leading to apoptosis [35]. The mitochondrial pathway of apoptosis is of particular interest here as it is often mutated in cancer cells. It has therefore been studied and modeled in several works [36–40]. They all intend to represent kinetic reactions between pro-and anti-apoptotic Bcl2 family proteins which may lead to mitochondria permeabilization and subsequent release of their content into the cytosol that would eventually trigger cell apoptosis.

2.3. Mechanistic Cell Population Models

Mathematical models of molecular pathways may then be linked to tissue level models in order to describe the influence of intracellular reactions on the cell population behavior. Mechanistic cell population model types range from cellular automata to ordinary and partial differential equations.

Physiological variability between individual cells can easily be represented by stochastic cellular automata models in which cells are simulated one by one, as individual agents. So-called individual-based models (IBMs) are amenable to include any kind of rules one puts in the cellular model. However, this kind of computational model does not allow mathematical analysis of asymptotic behaviors as they rather intend to investigate possible properties of the cell population. Alarcon *et al*. [41] used a cellular automaton model to represent tumor growth in a vascular environment, opening the way to the possible representation of anti-angiogenic therapies. Jagiella *et al.* also designed an IBM to study cancer cell population dynamics and assumed that cell entry into the cell cycle is governed by a phenomenological function which depends on glucose and oxygen extracellular concentrations [42]. Altinok and Goldbeter developed a cellular automaton in which cell cycle phases are explicitly represented [43–45]. Indeed, transitions between phases of the cell cycle are assumed to respect some prescribed rules. For instance, each phase is characterized by a mean duration associated to an inter-cell variability within the cell population.

Then, the simplest continuous models to represent cell population behavior over time are based on ordinary differential equations (ODEs). Basic ODE models for cancer growth are primarily the exponential, the logistic, and the Gompertz models, which have been studied in numerous works (see [46] for a review). However, these models consider a sole cancer cell population whereas it may be relevant to consider several populations to account for tumor heterogeneity. For instance, in [47, 48], authors assumed several tumor cell subpopulations characterized by their sensitivity to chemotherapy agents. Moreover, distinguishing between tumor and healthy cell populations enables to take into account possible side effects of treatments [49, 50]. As most anticancer drugs preferentially kill cells in a specific phase of the cell cycle, ODE-based models that integrate two or more compartments representing the phases of the cell cycle have been developed (reviewed in [46]).

To increase accuracy of cell population modeling, one may consider physiological features of cells and in particular cell age in the different phases of the cell cycle. To address this issue, physiologically structured partial differential equation (PDE) models which take as variables time together with the physiological age in cell cycle phases have been developed in numerous works [51–54]. Asymptotics behaviors of this kind of representations can be studied when the model is tractable, sometimes resulting in theorems. For instance, the McKendrick PDE framework is of particular interest as its asymptotics is governed by a first eigenvalue λ, also called the Malthus exponent. This means that its solution for large times is equivalent to a bounded function times $\exp(\lambda t)$.

To design an even more realistic model of tumor growth, one may include modeling of angiogenesis. This type of models allows optimizing co-administration of standard chemotherapy drugs and anti-angiogenic agents which usually act on endothelial cells thus inhibiting vessel formation. Hahnfeldt *et al.* [16] have represented

tumor growth using a Gompertz model in which the carrying capacity K undergoes variations overtime standing for spontaneous, tumor-induced and anti-angiogenic drug-induced effect on blood vasculature. In a more mechanistic way, Billy *et al.* designed a model of endothelial cells which act on cancer cell population through the extracellular oxygen concentration [55].

Once one has designed an appropriate representation of cell population dynamics, anticancer drug pharmacokinetics-pharmacodynamics modeling may be added. A simple formulation says that pharmacokinetics (PK) is the study of what the body does to the drug (e.g., metabolism, transport), whereas pharmacodynamics (PD) is the study of what the drug does to the body (toxicities/ therapeutic efficacy).

2.4. PK-PD Molecular Models: From Drug Infusion to Effects on Cell Death and Proliferation

In this part, we review existing physiologically based models of 5-FU, oxaliplatin and CPT-11 molecular PK-PD which are the three main cytotoxic drugs used in therapies against mCRC. Those models describe the drug pharmacology either at the level of a single cell, of a cell population or of the whole organism.

2.4.1. 5-fluorouracil (5-FU) Molecular PK-PD

5-FU intracellular molecular PK-PD has been modeled in a first work which focuses on relevant molecular mechanisms [9]. This system of ordinary differential equations computes the dynamics of protein and drug concentrations in the intracellular or the blood compartments (Fig. **2**). 5-FU and LV are infused in the plasma and reach the intracellular compartment where they trap the target enzyme thymidylate synthase (TS) into stable ternary complexes which irreversibly consumes free TS. More precisely, the intracellular active compounds, FdUMP for 5-FU and methylene tetrahydrofolate (MTHF) for LV, exert their action on TS by yielding first a reversible binary complex B binding 5-FU and TS, and then the irreversible ternary complex T by the adjunction of MTHF. Concerning PK, LV cell uptake is considered as passive and therefore not saturable whereas 5-FU enters the cells through a saturable mechanism. The intracellular form of 5-FU FdUMP is expelled outside of the cells by an ABC transporter whose expression is enhanced by drug exposure through the activation of a nuclear factor (Fig. **2**). The model also describes the degradation of 5-FU by hepatic dihydropyrimidine dehydrogenase (DPYD). Genetic differences in 5-FU catabolism by DPYD could be taken into account in this model by different Km and Vmax values of the enzyme activity [9].

Bodin *et al*. have implemented a multi-scale mathematical model of 5-FU PK-PD that links the drug injection into the blood to its efficacy on tumor growth by integrating its molecular pharmacokinetics and intracellular mechanism of action [56]. They have taken into account two different observation scales. At the cell level, they have modeled 5-FU-induced blockade of TS enzyme which inhibits DNA synthesis but also the incorrect incorporation of FdUTP to DNA leading to abnormal DNA production. At the tissue level, they have integrated the effect of those perturbations of DNA synthesis on cell cycle regulation and resulting tumor growth. A sensitivity analysis on TS level shows that it may be a potential biomarker regarding efficacy, as already reported in the clinical literature. This model also allows to simulate the effect of 5-FU on mCRC in order to test hypotheses to help optimizing treatments in particular by comparing different 5FU-based protocols in terms of efficacy.

Finally, Tsukamoto *et al.* have designed a whole-body physiologically based PK model of capecitabine, the oral prodrug of 5-FU, in cancer patients [57]. Four compartments have been considered: the liver, the gastrointestinal tract, the tumor and non-eliminating tissues (NET) such as skin and muscles. The mathematical variables of the model correspond to concentrations in each compartment of the parent drug capecitabine and of its three metabolites: 5'DFCR, 5'DFUR and the active one 5-FU. Enzyme kinetic parameters and plasma/tissue binding rates were inferred from *in vitro* data in human liver and intestinal cells. The model accurately predicted blood concentrations in cancer patients of all four compounds over time. A sensitivity analysis was performed on parameters to identify the ones that most influenced capecitabine PK. 5-FU blood concentration was most influenced by DPYD hepatic activity.

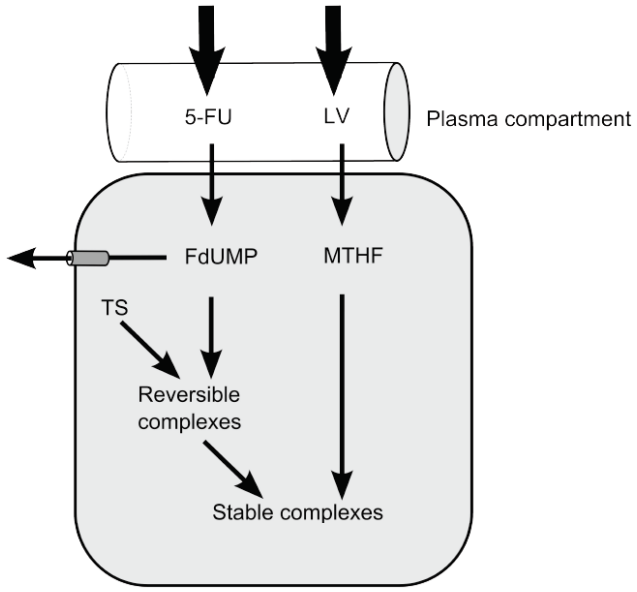

Fig. (2). Mathematical model of 5-FU molecular PK-PD [9] See text for details.

2.4.2. Oxaliplatin (l-OHP) Molecular PK-PD

Oxaliplatin PK-PD is rather simple as the drug is not metabolized and directly targets DNA. Indeed, it exerts its action on cells by creating irreversible oxaliplatin-DNA adducts that subsequently yield double-stranded breaks in the DNA. An ODE-based model of oxaliplatin PK-PD has been designed in which variables represented are concentrations, either in the blood, or in the tissues [49, 58, 59]. In this model, oxaliplatin is infused in the blood and may irreversibly bind to plasma proteins. The drug has been assumed to diffuse passively into the tissues. In the intracellular medium, oxaliplatin can either associate to free DNA (i.e., the drug target) or to reduced glutathione, that thus acts as a competitive drug inhibitor. In this model, DNA molecules which have been trapped by oxaliplatin may return to their free DNA state which represents the activity of excision repair enzymes. Apart from blood PK constants that are easily accessible, other parameters have been evaluated so as to produce likely behavior for the drug in tissues [59].

2.4.3. Irinotecan (CPT11) Molecular PK-PD

CPT11 molecular PK and PD have been studied in human colorectal adenocarcinoma Caco-2 cells which has led to the design of an ODE-based mathematical model [60]. Molecular pathways of CPT11 PK-PD are modeled according to biological data published in the literature and experimental results obtained in Caco-2 cells ([61], Fig. **3**). Briefly, CPT11 is bioactivated into SN38 through the variable CES representing the sum of all carboxylesterases activity. This enzymatic reaction is modeled by Michaelis-Menten kinetics. SN38 is deactivated into SN38G. This reaction also follows Michaelis-Menten kinetics in which the mathematical variable UGT stands for the sum of all UGT1As enzymatic activities. The intra-

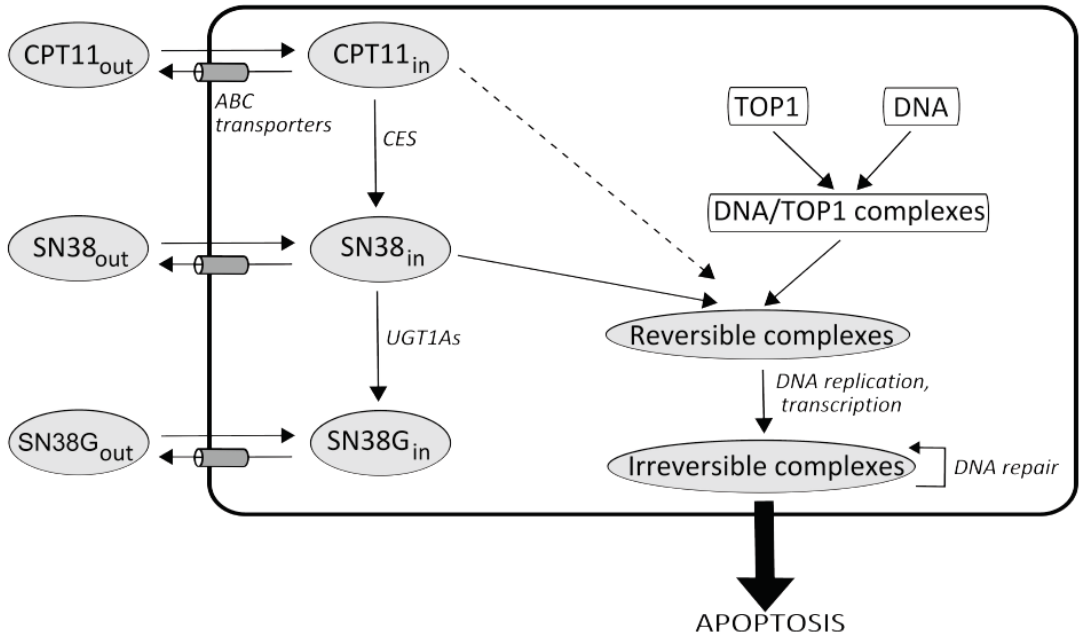

Fig. (3). Mathematical model of CPT11 molecular PK-PD [60] See text for details.

cellular uptake of CPT11, SN38 and SN38G is assumed to be passive and modeled as a free diffusion across a membrane. CPT11 and SN38 efflux are mediated respectively by ABC_CPT (mainly standing for the sum of activities of ABCB1, ABCC1, ABCC2) and ABC_SN (for ABCC1, ABCC2, ABCG2). Efflux follows Michaelis-Menten kinetics as experimentally demonstrated in the literature. Diffusion from inside to outside of the cells is neglected.

As regards its pharmacodynamics, CPT11 is an inhibitor of TOP1, an enzyme present in all nucleated cells [61]. The function of TOP1 is to relax DNA which may be supercoiled by several processes including replication and transcription. TOP1 binds to DNA and cuts one strand which is thus able to rotate around the molecule. Then TOP1 dissociates from DNA allowing the reconnection of the broken strand. CPT11 and its active metabolite SN38 prevent TOP1 religation by creating DNA/TOP1/drug complexes which can spontaneously dissociate but have a longer lifetime than DNA/TOP1 complexes. Collisions between those ternary reversible complexes and replication or transcription mechanisms convert them into irreversible covalent DNA damage which triggers DNA repair and possibly leads to cell cycle arrest and apoptosis. In the mathematical model, CPT11 ability to bind to TOP1 is neglected so that SN38 is the only molecule able to stabilize DNA/TOP1 complexes into DNA/TOP1/SN38 ones. Those ternary complexes are able to spontaneously dissociate or could be converted into irreversible complexes after collision with transcription or replication mechanisms (Fig. **3**). The amount of TOP1 complexes on the DNA is the output of the model since it has been experimentally correlated with CPT11 cytotoxicity both *in vitro* and in cancer patients [60]. Parameters of this CPT11 PK-PD model have been estimated from experimental data in Caco-2 cells combined with information from literature.

CPT11 is known to trigger the induction of ABC transporters, responsible for the efflux of the drug and its metabolites outside of the cells. Overexpression of those transporters prevents drug accumulation in the intracellular medium, therefore decreasing drug efficacy. A critical clinical concern lies in the design of CPT11-based therapeutic strategies which eradicate a maximum number of cancer cells despite their ability to become resistant. In order to address this issue, the above-mentioned model of CPT11 molecular PK-PD has been supplemented with a new model of CPT11-induced overexpression of ABC transporters [62]. The proposed molecular mechanism leading to ABC transporter induction consists in the activation by CPT11-induced DNA damage of nuclear factors which then promote the expression of ABC transporters. This assumption is consistent with experimental results in cancer cell lines which show that DNA double-stranded breaks resulting from CPT11 exposure activate the nuclear factor NF-κB which enhances ABCB1 expression. In the same way, chemical stress may activate the nuclear factor Nrf-2 which is known to promote the expression of ABCG2, ABCC1 and ABCC2 [62]. In the model, nuclear factor activation by CPT11 is phenomenologically represented by an S-shape function which shows a steep increase when DNA damage exceeds the induction threshold. Indeed, experimental results show that CPT11 induces a rapid and transient activation of the nuclear factor NF−κB which is dose-dependent and rapidly saturates when the dose of CPT11 is increased. This modeling choice results in a persistent overexpression of transporter mRNA and protein amounts which lasts after the drug exposure that is consistent with experimental literature as the reversal of transporter induction was observed only after two months in cultured cell lines [62].

Finally CPT11 PK-PD model at the tissue level has been adapted to build a whole-body physiologically based model for mice [63]. It is constituted of seven compartments which represent the simulated organs. The first compartment stands for the liver which plays a critical part in CPT11 metabolism. Then the two main toxicity targets of the drug which are the intestine and the bone marrow are modeled, as well as the blood, and the tumor to account for drug efficacy. Finally, the Non-Eliminating Tissue (NET) compartment represents all other tissues such as muscles or skin. CPT11 and its metabolites circulate in and out of the tumor, the bone marrow, the NET and the liver compartments through the blood circulation. Concerning the intestine, it is modeled by two compartments which represent the cells of the intestinal mucosa and the intestinal lumen. A bidirectional transport is assumed between the mucosa and the lumen. Moreover, the drug and its metabolites can be transported from the intestinal cells to the liver through the hepatic portal vein. The entero-hepatic circulation is modeled by a

drug transport from the liver to the intestinal lumen which stands for biliary excretion. Finally, renal clearance is modeled as degradation terms for CPT11, SN38 and SN38G in the blood compartment. The intestinal lumen compartment also presents degradation terms accounting for CPT11 and SN38 intestinal clearance, SN38G being exclusively eliminated through the kidneys. Each compartment contains an adaptation of the mathematical model of CPT11 tissue PK-PD [60]. Kinetics parameters of the model were partially estimated both from blood and tissue pharmacokinetics data in mice [63].

2.5. Linking PK-PD Models to Cell Population Level: Drug Targets

The above-described mathematical models of drug PK-PD simulate as computational events the molecular fates of the drugs from their infusion to their activities in the intracellular compartment. To bridge the gap between the molecular and functional effects of anticancer drugs, it is convenient to link those models with cell population representations which simulate the dynamics of the cell population in response to the molecular effect (Fig. **1**). In particular, it is of interest to describe how the intracellular drug activity affects cell death and proliferation, and how this is reflected at the cell population level (reviewed in [46]).

Cytotoxic drugs aim at killing cells, either by directly launching cell apoptosis, or inducing a blockage in a phase of the division cycle where long-term survival is impossible. The simplest way to model drug-induced cell death is a direct increase of death rates. However, many anticancer drugs are phase specific which justifies representing the four phases of the cell cycle in the population model. In age-structured models of the cell division cycle, where cell cycle phases are distinct, separated by transition rates, it is also possible, rather than enhancing death rates, to act on phase transitions (so-called checkpoints, mainly between G1 and S, and between G2 and M phases). We allude here at McKendrick-like PDE models in which inputs of drug may be considered as impacting different death terms in phases or transition rates between phases [46].

On the contrary, cytostatic drugs do not directly kill cells but rather slow down their proliferation by turning down molecular pathways which enhance the rate of entry into the cell cycle (e.g., Cetuximab which targets EGFR). Thus, cytostatic drug effect mainly keep cells in a quiescence state. A possible way to represent the effect of cytostatic drugs is to use age-structured models in which cell cycle phases are not necessarily detailed, keeping only one proliferative phase, and introducing one quiescent compartment. One may thus represent cytostatic effects by a contrasted fate at the end of mitosis, sending proliferating cells either back into the division cycle, or to a sideway representing a quiescent phase from which they cannot come back to proliferation [52]. From a theoretical point of view, such a model displays the advantage to be still linear and thus amenable to asymptotic analysis by investigating its first eigenvalue, or Malthus exponent.

Finally, one may want to represent the effects of anti-angiogenic drugs on cell population dynamics. There are a lot of models dedicated to specifically represent the action of these anticancer agents that do not act directly on the cancer cell populations themselves, but on their vascular environment. The representation of their effects obviously depends on the prior choice of a model concerning angiogenesis. Anti-angiogenic drugs have been considered in particular in ODE models [16, 64] and in PDE models, physiologically structured or not [55, 65-67]. In these models, they act by decreasing the "carrying capacity" of the tumor, or they choke progression in the cell cycle at the G1/S transition.

2.6. Circadian Control of Cell Physiology and Drug PK-PD

Most biological functions in mammals such as rest-activity, body temperature or hormonal secretions display rhythms of period between 20 and 28 hours called circadian rhythms [9]. This circadian organization induces variations in the toxicity and efficacy of many anticancer drugs with respect to their circadian time of administration and should therefore be taken into account in therapeutics optimization.

Circadian changes are coordinated by the suprachiasmatic nuclei (SCN), an endogenous pacemaker located in the hypothalamus. SCN functions display an intrinsic genetically-determined period which is entrained and calibrated at precisely 24 hours by environmental synchronizers such as the alternation of days and nights, socio-professional activities and meal timing. This central pacemaker controls the molecular circadian clock present in each nucleated cell through physiological signals. The cellular molecular clock is constituted of interconnected regulatory loops involving about 15 clock genes such as CLOCK, PER, BMAL, or REV-ERBα. Those genes display circadian rhythms in their expression and generate in turn circadian oscillations of various gene and protein amounts. In particular, many enzymes involved in drug metabolism, cell cycle, DNA repair or apoptosis display circadian variations and induce rhythms in the toxicity and efficacy of many anticancer drugs.

Circadian rhythms in mammals physiology result in variations in the toxicity and efficacy of many drugs with respect to their circadian time of administration, named chronotoxicity and chronoefficacy. Concerning mCRC, numerous clinical trials have compared chronotherapeutics administration schemes to their paired constant-rate infusion schedule lasting an integral multiple of 24 hours and involving the same drug doses [9]. In particular, two international randomized phase III trials have compared the chronomodulated scheme ChronoFLO5 to an equivalent constant delivery in 278 patients with mCRC. ChronoFLO5 combines the daily delivery of oxaliplatin over 11.5 hours with peak flow rate at 4: 00 p.m. and that of 5-FU/LV over 11.5 hours with peak flow rate at 4: 00 a.m., for 5 consecutive days. The other cohort of patients received the same doses of the same three drugs, at a constant rate over the same5-day span. In those trials, chronomodulated delivery reduced the incidence of grade 3-4 mucositis by fivefold and halved the incidence of peripheral sensory neuropathy [9]. A third randomized trial has compared the chronomodulated administration of the same three drugs over 4 days (ChronoFLO4) to a conventional constant-rate infusion over 2 days (FOLFOX2) in 564 patients with mCRC. The main endpoint of this study which was overall survival did not differ as a function of treatment schedule. However, the relative risk of an earlier death on ChronoFLO4 was significantly increased by 38% in women and decreased by 25% in men compared with conventional delivery. A recent meta-analysis of these three randomized trials involving 842 patients in total confirms that the chronomodulated infusion achieves similar or worse efficacy compared with conventional delivery in women. Conversely, in men, the ChronoFLO treatment significantly increases tumor response and survival compared with constant delivery, independently of all other prognostic factors. This result highlights the need for chronotherapeutic personalization in which chronomodulated administration schemes would be tailored according to the patient circadian and genetic profile. To address this issue, mathematical models of the circadian clock and its control on anticancer drug PK-PD have been designed.

First, several molecular models of the cellular circadian clock have been developed involving different levels of complexity [68–71]. In particular, Goldbeter *et al.* take into account the regulatory effects of clock genes and their proteins together with their post-translational regulation. Molecular interactions between the circadian clock and the cell cycle through the circadian control of Wee1 and p21 have been mathematically studied using ODE-based models [29, 72, 73]. The influence of circadian clock gene knockouts on the cell cycle has been studied to further validate the models [73]. Then, several approaches have been undertaken to model

cell proliferation and its circadian control at a cell population scale in the presence or absence of pharmacological control. Physiologically based PDE models taking into account the cell cycle phases have been extended to integrate the circadian control of death rates and cell cycle phase transitions [54, 74]. These models enable a theoretical study of cell proliferation under circadian control. Then, starting from these PDE-based models and with additional mathematical assumptions, delay differential equations can also be derived to model circadian-controlled cell proliferation [75]. An alternative approach involving agent-based models to simulate the cell behavior has also been proposed [43, 58]. Finally, chronoPK-PD models have been designed, at the level either of a single cell, of a cell population, or of the whole organism, for the three main cytotoxic drugs used in mCRC treatments: 5-Fluorouracil [9], oxaliplatin [49] and irinotecan [60, 63]. These ODE-based molecular models integrate circadian variations of genes and proteins involved in the PK-PD of the different drugs at stake.

3. MATHEMATICAL APPROACHES TO OPTIMIZATION OF MCRC TREATMENTS

3.1. Pitfalls Encountered in the Clinic: Unwanted Toxic Side Effects and Drug Resistance in Tumors

How to differentiate the responses to treatments of healthy tissues from those of tumors, so as to avoid being more (or at least not less) harmful than beneficial to patients? And how to predict and avoid evolution of cancer cell populations towards drug resistance? These are the two main problems faced by clinicians in the treatment of cancers that limit the use of increasing drug doses. Note that it is a general problem in therapeutics that involves, mutatis mutandis, other diseases characterized by uncontrolled proliferation of an aggressive population of agents that live at the expense of normal cell populations in an organism, in particular in the field of antibiotherapy, virology and parasitology.

This problem has often been considered from an only static point of view, taking into account upper limits for drug doses, and lower limits for between-courses intervals, supposed to protect the patient from toxic adverse effects, or taking as objects of study two different given cancer populations, a drug sensitive one and a resistant one. However, we advocate here for a more dynamic and continuous approach, considering simultaneously the instantaneous effects of anticancer drugs on healthy tissues and on tumors for the toxicity issue on the one hand, and on the other hand drug resistance to a given drug in a cancer cell population as a continuous phenotypic trait structuring the population, amenable to evolution under the environmental pressure of a drug or of a combination of drugs.

The toxicity issue requires identification of clear differences between healthy and cancer cell populations, and it has been proposed to take advantage of different behaviors of control of proliferation and of pharmacodynamics by circadian clocks in these two populations. This has led to different studies [44, 45, 49, 54, 59], involving or not the cell division cycle, according to physiological considerations, but not with actual molecular targets. These studies are reported below in the Section Circadian chronotherapeutics. As regards the drug resistance issue, it has been considered from a molecular point of view, as mentioned in the Section *Approved chemotherapy regimens against mCRC* and reviewed in [7]. From the point of view of evolution towards resistance, following a Darwinian vision, it has been studied using a phenomenological (ecological-like) population dynamics model structured according to a phenotypic trait in [76] with simulations representing the effect on proliferation in cell populations of cytotoxic and cytostatic drugs in general.

3.2. Resistance Due to ABC Transporter Overexpression

Tumor cells may become resistant to anticancer agents after prolonged exposure. Those drug-induced molecular changes or mutations may highly modulate the drug activity and should therefore be taken into account in therapeutics optimization. In particular, multidrug resistance (MDR) is characterized by the ability of cancer cells to become simultaneously resistant to many anticancer drugs. A possible cellular mechanism of MDR is the drug-modulated induction of ATP-Binding Cassette (ABC) transporters which actively pump molecules outside of the cells. The enhancement of ABC transporter expression in tumor tissues prevents anticancer drugs from accumulating in the intracellular medium and therefore decreases their efficacy.

To the best of our knowledge, three published works propose mathematical models for ABC transporter induction by anticancer drugs. A mathematical model of doxorubicin PK-PD includes transporter overexpression which is assumed to be directly proportional to the intracellular drug concentration [77]. However, this modeling assumption does not render an account of the experimentally observed threshold on drug concentrations above which resistance is triggered. Moreover, this model allows the quantity of ABC transporters to grow to infinity in the case of large drug concentrations, which is not the case in the two following studies.

Another work models the molecular PK-PD of 5-FU and includes the drug-induced transporter over-expression [9]. In this model, the nuclear factor remains activated as long as the intracellular drug concentration exceeds an induction threshold. However, this may not be experimentally accurate since NF $-\kappa$B kinetics in the presence of CPT11 consists in a transient activation of few hours which vanishes before the drug removal [62]. As regards to optimization of drug administration, this modeling choice implies that killing a maximum number of cancer cells would be achieved by an exposure to a dose below the induction threshold during a long period in order not to trigger the resistant mechanism.

Finally, in the third study which focus on CPT11 PK-PD, authors assumed that ABC transporter overexpression results from the activation by drug-induced DNA damage (and not directly by drug concentration as in [9]) of nuclear factors which then promote the expression of ABC transporters. Authors then theoretically optimized exposure to CPT11 given as a single agent or combined either with ABC transporter inhibitors, or with inhibitors of nuclear factors. Considering a sole cancer cell population endowed with the ability of inducing their transporters, they concluded that, for any drug combination, the highest concentration of CPT11 should be administered in order to kill a maximum number of cancer cells, despite the triggering of resistance [62]. On the contrary of the model published in [9], this model concludes that the resistance mechanism is going to be triggered anyhow and that the highest tolerable dose should be given during the first cell exposure in order to kill a maximum number of cancer cells before they overexpress their transporters and become resistant. Then authors considered a population of healthy cells which were assumed to be identical to cancer cells except that they were not able to become resistant. Optimal schemes were defined as the ones which maximized DNA damage in cancer cells under the constraint of DNA damage in healthy cells not exceeding a tolerability threshold. The optimal therapeutic strategy consisted in combining CPT11 with ABC transporter inhibitors as it achieved a complete reversal of resistance by means of the lowest concentrations of CPT11 [62].

3.3. Optimization: From Investigation on Grids to Optimal Control

Optimization of cancer treatments may be understood in different ways: it may be quite intuitive, based on graphical estimation by varying parameters on grids, more rational and less visual when using numerical optimization algorithms, but also in the ideal case completely analytical, using theorems that yield the proven optimal solution when it is possible, which is not always the case. Whatever theoretical it may be, it always aims at decreasing the number of tumor cells, if possible eradicating them, but the optimization prob-

lems considered differ according to the constraints taken into account: limiting toxic side effects on healthy cell populations, or, less frequently because mechanisms of drug resistance are multiple and not easy to represent, avoiding the development of resistant subpopulations of cancer cells.

In the case of toxicity constraints, L^2 or L^1 criteria (with optimization methods leading to more complex analysis in the case of L^1 criteria) have been used in optimization procedures, and the constraints on cell populations are often fixed, for instance to an a priori optimal average of the healthy cell population number [59]. However, in the perspective of continuous infusion treatments, it is natural to simultaneously take into account healthy and cancer cell population numbers, i.e., to design optimal infusion strategies as a time sequence of instantaneous trade-offs between maximal tumor cell kill and minimal healthy cell kill, i.e., using L^∞, rather than L^2 or L^1, criteria. This point of view is developed in particular in [49], where it is shown that, provided that reasonable hypotheses are satisfied, the searched for maximum or minimum (in this case, more precisely, the minimum of a Lagrangian) is a differentiable function of the infusion profile.

Graphical optimization, performed by simulations, is a possible first approach used when attempting to solve an optimal control problem; it may be performed by varying parameters of a given set of control functions representing here anticancer drug infusion flows in PK-PD models: [44, 45, 78], but it soon finds its limits.

Numerical (algorithmic) optimization methods take into account the effects of drugs on both healthy (to be preserved) and cancer (to be hit as hard as possible) cell populations. In [79, 80], the authors, starting from G.W. Swan's works [85] on optimal control applied to medicine, represent toxicity constraints on a healthy cell population (supposed to be the hematopoietic tissue) by both constant upper bounds on the drug concentrations and by constant lower bounds on the healthy cell population, and propose optimized drug delivery protocols. The same point of view is also presented in [49, 54, 59], designing a Lagrangian, linear combination between the objective function on cancer cells and the constraint function on healthy cells, both continuous functions [49, 54, 59], and the Uzawa or the Arrow-Hurwitz algorithms to compute gradients and yield numerical solutions to the optimization problem, which are optimized drug delivery flows as functions of time. The objective and constraint functions are cell population densities [49] or their growth exponents [54, 59].

On more theoretical grounds (i.e., showing theorems that lead to actually analytic solutions, rather than proposing numerical optimization schedules that are shown to converge numerically, but do not elicit the solutions as a function of the parameters of the problem), Kimmel and Swierniak [81], and Ledzewicz, d'Onofrio and Schättler (see e.g., [82]), the latter group working on Hahnfeldt's model of tumor growth with angiogenesis [64], controlled by both a cytotoxic and an antiangiogenic drug, have proposed optimal drug infusion strategies justified from a theoretical point of view. Such theoretical optimal solutions may then become benchmarks for practically more feasible computations, that in principle lead to only suboptimal solutions, as shown in [83, 84]. Note also that earlier works on optimal control by Swan [85] and by Costa and Boldrini [86, 87], using a theoretical point of view, take into account both the toxicity and the resistance (with a focus on the latter) constraints.

3.4. Optimizing Circadian Chronotherapeutics

The circadian organization of living organisms under therapeutic control modulates the response to anticancer drug depending on their time of administration. Circadian clocks exert their influence both on the target cell populations (e.g., by controlling cell cycle checkpoints [88]) and drug metabolism enzymes. The chronotherapeutic optimization problem can be formulated as the definition of objective (e.g., maximization of therapeutic efficacy on cancer cells) and constraint functions (e.g., tolerability thresholds). This is illustrated in a proof of principle study of in silico chronotherapeutics with oxaliplatin in [9, 49]. Parameter identification is performed on tumor growth curves in mice, with a simplified PK-PD model based on the jejunal toxicity and the antitumor efficacy of oxaliplatin. The solution to the optimal control problem is a theoretically optimized non trivial drug infusion flow. The treatment constraints critically determine the optimal chronotherapeutics schedule. Interestingly, constant rate infusions always achieve worse therapeutic outcomes than optimized time-scheduled regimens in these models.

Another study addresses the optimization of CPT11 circadian delivery in human cultured cells [60]. The above mentioned data-calibrated model of CPT11 PK-PD was used in numerical optimization procedures to compute theoretically optimal exposure schemes for Caco-2 cells. Cells synchronized with a seric shock were considered as healthy cells and non-synchronized cells as cancer ones as the circadian organization is often disrupted in tumor tissues [9]. The adopted therapeutic strategy consisted in maximizing DNA damage in cancer cells under the constraint that DNA damage in the healthy population remained under a tolerability threshold. They considered administration schemes in the form of a cell exposure to an initial extracellular concentration of CPT11, over 1 to 27 hours, starting at a particular circadian time (CT). For all considered doses, the optimal exposure scheme consisted in administering CPT11 over 3 hours 40 to 7 hours 10 starting between CT2h10 and CT2h30 which corresponded to 1 hour 30 to 1 hour 50 before the nadir of carboxylesterases (CES) protein amount. The optimal schemes were not centered on the nadir of rhythm but rather extended after it, when UGT, ABC_CPT and ABC_SN amounts were higher and therefore protected more effciently healthy cells. For any maximum allowed toxicity, the optimal duration did not exceed 7 hours 10, highlighting the need for short exposure durations to optimally exploit the temporal difference between healthy and cancer cells. Regarding efficacy, those optimal schemes induced twice as DNA damage in cancer cells as in healthy ones. A clinical interpretation can be obtained by rescaling to 24 hours those results for Caco-2 cells that displayed a period of 26 hours 50. Thus, an optimal administration of CPT11 to cancer patients should result in the presence of the drug in the blood during 3 hours 30 to 6 hours 30, starting 1 hour 30 to 1 hour 40 before the minimum value of CES activity in the patient.

3.5. Metronomic Therapies

The words 'metronomic therapy' were coined in a 2000 article by D. Hanahan [89] under the suggestive first words of its title stating that 'less is more, regularly', meaning by this that rather than giving high doses of anticancer drugs during a short period of time followed by a long 'recovery' interval, it is better to deliver, for a given total delivered dose, small quantities of a drug on a regular time schedule, without long interruptions in the chemotherapy course. It has firstly found a rationale based on inhibition of tumor neoangiogenesis, but more recently other hypotheses have been proposed, relating its successes to other causes, such as activation of the immune response or induction of tumor dormancy [90–92]. It might also be related to the concept of adaptive therapy, advocated by R. Gatenby [93], according to which the tumor, seen as a heterogeneous population of cells obeying evolutionary principles, should be controlled in its development (and not eradicated) by delivering limited drug doses in a regular way, so as to maintain inside the tumor a bulk of drug-sensitive tumor cells, supposed to contain the development of a drug-resistant tumor cell clone, that would otherwise fill the whole tumor and lead the treatment to certain failure.

The biological rationale for this therapeutic strategy [64] is usually (and has initially been) presented as efficiently limiting tumor neoangiogenesis, which thrives during treatment interruptions, allowing tumors to regrow even stronger, which leads treat-

ments to failures. Note that metronomic therapies are also supported by another recent model [94] based on a previous multiscale physiological model of tumor growth with angiogenesis [55]. It is also noteworthy that on different bases, without involving neoangiogenesis, but in a chronotherapeutic setting, other theoretical studies [59, 74] have also led to the conclusion that avoiding treatment interruptions during long time intervals, but on the contrary administering a chemotherapy on a 24 hour-periodic basis with a short actual drug infusion time during this 24 hour-period, is an optimal (in fact only suboptimal, in as much as in its principle it searches for necessary, but not sufficient, conditions of optimality) strategy.

A theoretical study of the involvement of tumor neoangiogenesis together with the influence of circadian clocks in combined treatments has not been done so far, to our knowledge; however, note that in the now classical metronomic therapy setting, theoretical optimal control strategies combining cytotoxic and antiangiogenic drugs have been proposed by d'Onofrio, Ledzewicz and Schättler [82], as already mentioned in the *Optimization* section.

4. CONCLUSIONS AND FUTURE PROSPECTS

Current clinical chemotherapy against mCRC involves combinations of several drugs that have different mechanisms of action. Taking into account drug interactions between cytotoxic agents has not been done so far, to our knowledge, in molecular models. Yet, since these drugs use the pathway of the general blood circulation, often linking -irreversibly or not -to plasma transport proteins, their blood pharmacokinetics may be affected by such interactions, be it only in a competitive way, to be transported towards peripheral tissues or eliminated via biliary or urinary excretion. This remains a challenge to design precise qualitative and quantitative whole-body PK models.

Although it has not been done so far to our knowledge, the study of combination of cytotoxic and cytostatic drugs is of particular interest in the case of mCRC. It is possible to add a representation of cytostatic drug activity in age-structured models with targets for cytotoxic drugs acting on death rates. Indeed, reversing the consideration of the effects of cytotoxic drug treatments on cell cycle phase transitions as presented in [54] to mainly cytostatic effects, in as much as they do not directly impact death rates, it is possible to use these two different kinds of targets in the same physiologically structured cell population model. This can be done by a simple model as the one proposed by Gabriel *et al*. [52], in which cytostatic effects (in this case, identified as erlotinib acting on non-small cell lung carcinoma PC-9 cells in culture) consist in sending proliferating cells (in G1 -phase if one considers cell cycle phases) to a sideway (= the quiescent G0 -phase) from which they cannot come back to proliferation, this effect being added to a model in which cytotoxic effects are represented by a direct action on death rates, as proposed in [59]. Representations of combinations of drugs exerting their actions on both targets: death rates for 5-FU, oxaliplatin and irinotecan, G1 /S phase transition for growth factor inhibitors (mainly monoclonal antibodies today in the clinic, but also tyrosine kinase inhibitors and possibly CDK inhibitors), should thus benefit from age-structured models involving combinations of PK-PD systems with output on both these modeled targets in healthy and cancer proliferating cell populations.

Several efforts have been undertaken to personalize medicine based on gene polymorphisms, so called pharmacogenomics. In the case of mCRC, UGT1A1 polymorphism may predict severe toxicity of CPT11 [95]. Here, gene mutation is directly correlated to changes in UGT1A1 activity. However, over the last decades, only few gene polymorphisms have showed clinical relevance which might be explained by the lack of correlation between single nucleotide polymorphisms with molecular activities [21]. Therefore, to allow personalized medicine, one should advocate phenotypic measurements in patients prior to any treatments.

Numerous studies are in progress to allow patient-tailored therapies based on measurements before drug administration. For instance, the CIME cocktail is designed to assess the activity of major drug metabolism pathways in patients [96]. The cocktail is composed of several chemicals that are substrates for the main metabolism pathways. Once the cocktail is administered, 20 substrates and metabolites are measured in the patient's plasma which allows quantitatively assessing the different metabolic rates. Another kind of studies consists of cell culture of patients' samples. For instance, the expression and activity of TOP1, the drug target of CPT11, have been assessed in patient's healthy and cancer intestinal tissues [97, 98].

Another type of phenotypic measurements concerns the monitoring of biological rhythms of the patient over 24 hours, so called circadian rhythms. Minimally- or non-invasive procedures are nowadays available to provide high quality and reliable data about the patient circadian clocks and their coordination [9]. Frequent sampling over several days provides an insight into the Circadian Timing System (CTS) of the patient. Chronobiology rhythms can be measured in patients in several ways. Rest-activity rhythms can be monitored through actimetry which has been considered as the method of choice regarding reliability, convenience and continuity in recordings [9]. Then temperature rhythms can be non-invasively assessed using different devices. Salivary samples may also be collected in order to measure gene expression levels in the oral mucosa, those of cortisol and melatonin being considered as relevant circadian biomarkers in cancer patients [9]. Moreover, cell culture of patient's samples may allow the determination of the patient's intrinsic period length: Brown *et al*. have determined the period length of 19 humans using cell culture of fibroblasts from skin samples [99]. The average value from all subjects was found equal to 24.5 h which closely matches reported average values for human circadian physiology in the absence of external synchronizers.

In conclusion, what should actually personalized medicine consist of, in particular for colorectal cancer therapies, and how is it related to treatment optimization? Although promising in its principles, pharmacogenomics may not be enough to draw personalized indications of drug delivery schedules in the clinic. Beyond companion diagnostics based on -very few -genotypes, it seems at least as necessary to use phenotypical characterizations of patient profiles, as proposed, e.g., by Meyer *et al*. [21] in a recent review article in which in particular the relationship between genotype and phenotype is examined in details. Personalizing anticancer treatment on a molecular basis should consist in optimizing the therapy according to the gene expression profiles of healthy and cancer cells of the patient and to their phenotypes, relevant for different 'omics' that do not all depend on gene expression. Molecular differences between normal and tumor tissues may be exploited in all these 'omics' dimensions in order to maximize the treatment efficacy and minimize its toxicities. Personalization of a given treatment ought to be achieved by characterizing a patient's profile with respect to its response to the drugs at stake, then optimization of the drug delivery schedules, should be adapted to the patient by determination of relevant individual parameters. Indeed, in the case of colorectal cancer as in the case of most complex treatments in medicine, the aim of physiologically based mathematical models is to take into account relevant physiological characteristics of patients, so as to individualize optimized general treatments, i.e., optimized on a parametric basis amenable to be adapted to instances of actual patients. Actually personalized medicine must consist of such conjunction of theoretically optimized treatments on physiological bases with their tailoring to individual patients, if we want it to make real sense.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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