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HYBRID MODEL OF ERYTHROPOIESIS AND LEUKEMIA TREATMENT WITH CYTOSINE ARABINOSIDE

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Abstract. A hybrid model of cell population dynamics, where cells are discrete elements whose dynamics depend on continuous intracellular and extracellular processes, is developed to simulate the evolution of immature red blood cells in the bone marrow. Cell differentiation, self-renewal or apoptosis are determined by an intracellular network, based on two proteins Erk and Fas and described by ordinary differential equations, and by local extracellular regulation performed by Fas-ligand, a protein produced by mature cells whose concentration evolution is represented by a partial differential equation. The model is used to study normal and leukemic red blood cell production (erythropoiesis), and treatment of leukemia. Normal cells are supposed to have a circadian rhythm, that influences their cell cycle durations, whereas leukemic cells, apart from being characterized by excessive proliferation and insufficient differentiation and apoptosis, are supposed to escape circadian rhythms. We consider a treatment based on periodic administration of Ara-C, an anti-cancer agent targeting cells in DNA synthesis. A pharmacodynamic/pharmacokinetic model of Ara-C is then proposed, and used to simulate the treatment. Influences of the period of the treatment and the day delivery time on the outcome of the treatment are investigated and stress the relevance of considering chronotherapeutic treatments to cure leukemia.

Key words. Hybrid model, leukemia treatment, chronotherapy, regulatory networks, cell cycle

AMS subject classifications. 92C30, 92C37, 92C50, 70F40, 68U20, 35Q70, 35Q92

1. Introduction.

1.1. Regulation of normal erythropoiesis. Haematopoiesis is the process by which immature cells proliferate and differentiate into mature blood cells. It starts with very few primitive haematopoietic stem cells and ends up with a huge number of mature erythrocytes (red blood cells), leukocytes (white blood cells) and platelets. We are concerned, in this work, with the red blood cell lineage, through the process of production and regulation of red blood cells, called erythropoiesis. The whole process of erythropoiesis happens in the bone marrow where erythroid progenitors, immature blood cells with abilities to proliferate and differentiate, divide to produce erythroblasts (mature progenitors), then reticulocytes (precursor cells) and finally mature erythrocytes (red blood cells), which leave the bone marrow and enter the

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blood flow.

Erythropoiesis occurs in discrete anatomic units of bone marrow, the erythroblastic islands. They consist of one or two macrophages surrounded by erythroblasts at different maturation stages [7]. Direct contact with macrophages promotes erythroblast proliferation. In vitro, erythroblasts proliferate 3-fold more in the presence of macrophages than erythroid progenitors cultured alone [7]. The inner erythroblastic layers of erythroblastic island contain immature cells, whereas the most mature cells are located at the periphery of the island.

Erythropoiesis is a robust system able to keep the number of circulating red blood cells at a constant level and to respond rapidly to restore that equilibrium following e.g. injury. This is done by regulating the fate of individual cells: proliferation (or self-renewal, the ability to produce cells with the same maturity level than mother cells), apoptosis (programmed cell death) and differentiation (the ability to produce cells more mature than the mother cell). Cell fate is determined by internal (gene regulatory networks) and external mechanisms involving growth factors, cell-cell interactions and other signals that are sensitive to the context of erythropoiesis. Then, internal and external regulations form multiple positive and negative feedback loops that maintain the integrity of the haematopoietic system [9, 10, 11, 15].

The hormone erythropoietin (Epo) is an important regulator of erythropoiesis and acts on erythroid progenitors by increasing proliferation, speeding up progression through maturation and protecting cells from apoptosis [20]. Epo is produced by the kidneys and released in the bloodstream, and brought to the bone marrow, due to hypoxia (lack of red blood cells). Cell-cell interaction within erythroblastic islands also plays an important role in erythropoiesis. Fas/Fas-ligand binding, where Fas is a membrane protein and Fas-ligand its ligand, contributes to the regulation of apoptosis of immature erythroid progenitors. Fas-ligand is produced by reticulocytes and stimulates apoptosis of immature erythroid progenitors [14]. However, increased level of erythropoietin acts in the opposite way and protects immature erythroblasts from apoptosis.

Growth factors (Epo for instance) trigger intracellular protein activation through transmembrane receptors located on cell surface. Several proteins are involved in either erythroid cell proliferation, differentiation or apoptosis. Figure 1.1 shows a simplified description of the regulatory network that controls erythroid progenitor fate [29]. Two intracellular proteins, Erk and Fas, are thought to be determinant for the regulation of self-renewal, differentiation and apoptosis. Erk induces erythroid progenitor self-renewal, whereas Fas induces both differentiation and apoptosis. As mentioned above, Fas-ligand (denoted by FasL in Figure 1.1) activates Fas; Epo influences Erk activation and shows an antiapoptotic effect through inhibition of caspases; Glucocorticoids (GC in Figure 1.1), growth factors associated with cell proliferation, act on Erk activation.

Hence, erythropoiesis appears as a very complex process, involving both an intracellular regulation through activation of key proteins, and an external regulation, based on growth factor activation or inhibition of proteins and on cell-cell interactions. Disorders in erythropoiesis can be due either to deregulation of the intracellular regulation networks or to dysfunctions in cell-cell interactions. Such disorders can cause, for instance, leukemias.

1.2. Treatment of acute myeloid leukemia with Ara-C. Leukaemia is a malignant disease characterized by abnormal proliferation of immature blood cells or haematopoietic stem cells within the bone marrow. These cells finally enter and

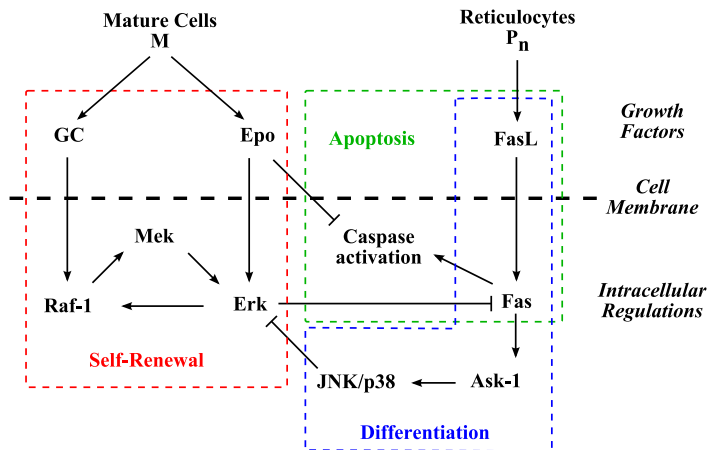
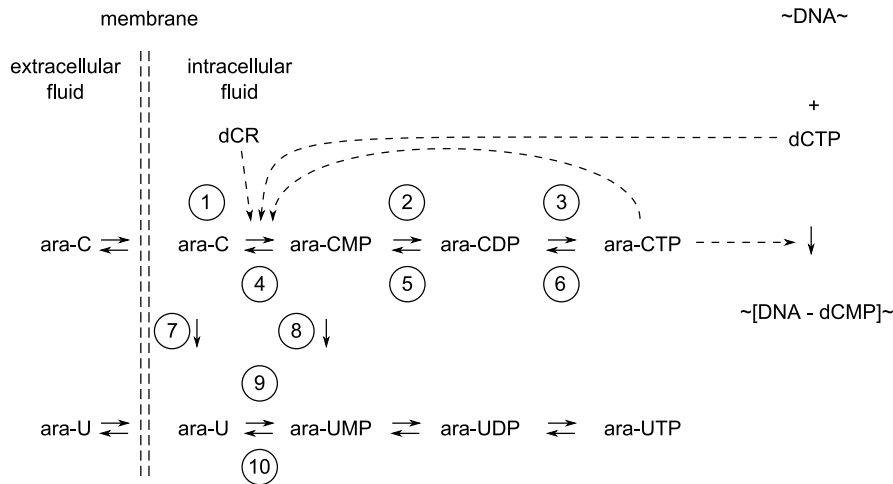


FIG. 1.1. Summary of intracellular protein interactions that determine erythroid progenitor fate, partially adapted from Rubiolo et al. [29].

invade bloodstream. There are four types of leukemia: myelogenous and lymphocytic (according to the haematopoietic lineage involved in the disease), each of which being acute (rapid increase of immature blood cells) or chronic (excessive production of relatively mature blood cells). We focus our attention on the acute myeloid leukemia (AML).

According to the French-American-British (FAB) classification, AML has eight different subtypes. In this work we concentrate ourselves on erythroleukemia, designated by M6 in the FAB classification. Erythroleukemia was first described by Di Guglielmo in the early twentieth century [22], it is characterized by a proliferation of erythroid and myeloid (white cells) progenitors, and sometimes a pure erythroid proliferation is observed [19]. The origin of erythroleukemia lies in a destabilization of erythropoiesis at the immature level, that is at the erythroid progenitor level. A dysfunction of the Epo-mediated apoptosis regulation has been stressed to be a causative agent of erythroleukemia. Evidences for self-activation of Epo-receptors at the surface of erythroid progenitors [13] could explain the sudden proliferation of immature erythroid cells. Yet, experiments conducted by [21] indicated that autocrine stimulation by erythropoietin in mice could result in intense proliferation of erythroid progenitors but never triggered erythroleukemia. Self-activation of Epo-receptors could not then explain appearance of erythroleukemia without considering other signaling pathways (for instance, including Erk or Fas) destabilization.

During past decade the first line therapy for AML patients was daunorubicin or idarubicin in combination with cytosine arabinoside (Ara-C), yet nowadays new investigational options exist [18]. In this paper we consider the chemotherapeutic treatment based on Ara-C. This drug is characterized by a short half-life and targets cells during DNA synthesis (S-phase of the cell cycle). After intravenous administration, the drug is rapidly metabolized, by deamination in the liver and kidney, to its inactive form uracil arabinoside (Ara-U). When in the bone marrow, it penetrates proliferating cells membrane and it can be transformed into its active form arabinoside triphosphate (Ara-CTP), which participates in the DNA duplication, replacing natural nucleotides. When the proportion of Ara-CTP in the DNA becomes sufficiently high, the cell dies by apoptosis. Metabolism scheme of Ara-C is presented in Figure

FIG. 1.2. *Metabolism of Ara-C*

1.2.

Ara-C acts on all proliferating cells whether they are leukemic or normal. Therefore, the aim in optimizing drug administration schedule is to increase cytotoxicity for leukemic cells and tolerance for normal cells. One possible approach to this problem is based on chronotherapy, which takes into account the small differences in the temporal organisation of the cell cycle between normal and leukemic cells.

1.3. Chronotherapy. Chronotherapy signifies drug delivery synchronized with biological rhythms in order to optimize the therapeutic outcomes. Cell physiology is regulated along the 24-h time. The suprachiasmatic nucleus, a hypothalamic pacemaker clock and at least 12 circadian genes act to generate and coordinate biological rhythms. The aim of anti-cancer chronomodulated treatment is to decrease toxicity and improve efficacy of the treatment. The same temporal pattern of phase-specific drug administration should have minimum cytotoxicity toward population of normal cells and at the same time should show high cytotoxicity toward cancer cells [1, 24].

The goal, in this work, is to use numerical simulation of a hybrid model (see Section 2.1) to improve chronoefficacy of S-specific Ara-C in the treatment of AML, when, contrary to leukemic cells, not damaged erythroid progenitors show specific daily variation in their DNA synthesis activity. After 24 hours studies of healthy bone marrow cells, circadian stage-dependent variations in the proliferative activity were demonstrated [30]. It was shown that, on an average, the percentage of total bone marrow cells in DNA synthesis phase was greater at midday than at midnight. Myeloid and erythroid precursor cells correlate positively and indicate a circadian temporal influence on S-phase of total bone marrow cells by displaying its acrophase at 1 p.m. [31]. Disruption of circadian rhythm in chronic myeloid leukemia (CML) was shown by analysing peripheral blood from healthy individuals and patients with CML for the expression of the nine circadian genes. The expression levels of six genes were significantly impaired in both chronic phase and blastic crisis of CML cases compared with those in healthy individuals [32]. This difference in populations of normal and leukemic cells allows us to expect to have maximal efficacy and minimal toxicity by treating patients at different times of the day. Therefore, we would expect that the development of a cell cycle phase specific cancer chronotherapy treatment

would depend on each single individual because of high variation in the time of their activity.

2. Mathematical model and methods.

2.1. Hybrid models. We developed a hybrid model of erythropoiesis with off-lattice cell dynamics [17, 26]. In this approach, cells are considered as discrete objects. They can interact with each other and with the surrounding medium mechanically and biochemically [12]. Cells have the ability to move, grow, divide, differentiate and die by apoptosis. The fate of each cell is determined by intracellular and extracellular regulatory networks. Intracellular regulatory networks are described by ordinary differential equations:

$$(2.1) \quad \frac{du_i}{dt} = F(u_i, u),$$

where u_i is a vector of intra-cellular concentrations of cell i , u is a vector of extra-cellular concentrations, F is a vector of reaction rates which is specified for each particular application.

The extracellular regulatory network [27] is described by partial differential equations:

$$(2.2) \quad \frac{\partial u}{\partial t} = D\Delta u + G(u, c),$$

where c is the local cell density, G is the rate of consumption or production of different species by cells, which will be specified in the following section, D is the extracellular diffusion rate.

We consider cells as elastic balls. Under the assumption of small deformations, we can express the force acting between them as a function of the distance between their centers. Thus, the force between two particles with centers at x_i and x_j is given by a function $f(d_{ij})$ of the distance d_{ij} between the centers. It is zero if the distance is greater than the sum of their radii. To describe the motion of a particle, we should determine the forces acting on it from all other particles and possibly from the surrounding medium. We describe the motion of each cell by the displacement of its center by Newton's second law:

$$(2.3) \quad m\ddot{x} + \mu m\dot{x} - \sum_{j \neq i} f(d_{ij}) = 0,$$

where m is the mass of the particle, the second term in left-hand side describes the friction by the surrounding medium, the third term is the potential force between cells. The force between two spherical particles is considered in the form

$$(2.4) \quad f(d_{ij}) = \begin{cases} K \frac{d_0 - d_{ij}}{d_{ij} - d_0 + 2H_1}, & d_{ij} < d_0, \quad j = i \pm 1, \\ 0, & d_{ij} \geq d_0 \end{cases}$$

where d_0 is the sum of cell radii, K is a positive parameter, and H_1 accounts for the compressible part of each cell. The force between the particles tends to infinity when d_{ij} decreases to $d_0 - 2H_1$. On the other hand, this force equals zero if $d_{ij} \geq d_0$. More detailed discussion of the model and various test cases and examples are presented in [5, 6].

In our model we consider two types of cells, erythroid progenitors, located in the center of erythroblastic island (the computational domain), and reticulocytes, located at the periphery of the island. Reticulocytes produce Fas-ligand which diffuses into extracellular matrix and influences erythroid progenitors.

2.2. Intracellular and extracellular regulation of normal erythropoiesis.

Intracellular regulation. We focus our attention on two key intracellular proteins, Erk and Fas, which are involved in cell fate decision (high levels of Erk induce cell self-renewal, whereas high levels of Fas induce either cell differentiation or apoptosis). Evolution of the concentrations of these proteins inside an erythroid progenitor is described by a system of two ordinary differential equations [11],

$$(2.5) \quad \begin{cases} \frac{dE_i}{dt} &= (\alpha(Epo) + \beta E_i^k)(1 - E_i) - aE_i - bE_i F_i, \\ \frac{dF_i}{dt} &= \gamma(FasL)(1 - F_i) - cE_i F_i - dF_i, \end{cases}$$

where E_i and F_i are the concentrations of Erk and Fas, respectively, in the i -th cell (numbering of cells is related to the discrete off-lattice model). The protein Erk is assumed to self-activate, this is described by the term E_i^k with $k > 0$, and to be activated by Epo, through the function α . When more Erk is activated, less Erk receptors remain free and, thus, activation rate saturates. In a similar way, Fas activation by Fas-ligand (whose concentration is denoted by $FasL$) and saturation are represented by the first term in the right hand side of the second equation in (2.5). This system also takes into account the fact that the self-renewal loop and the apoptosis/differentiation loop inhibit each other (see Figure 1.1). The corresponding terms are proportional to the product $E_i F_i$, according to the mass action law. Finally, both proteins E_i and F_i can be degraded, with constants a and d .

Parameters a, b, c, d are assumed to be positive. The function γ is a function of $FasL$, with $\gamma(FasL) = \gamma_0 + k_\gamma FasL$. From now on, and throughout this manuscript, the concentration of Epo is assumed to be constant, so the function α in (2.5) will be considered as a positive parameter. For this study, we focus only on the feedback control exerted on progenitor cells by reticulocytes through Fas-ligand (see the next paragraph *Extracellular regulation*).

This system of equations can have between one and three stationary points [11], depending on parameter values. The choice of parameter values is an important issue when describing intracellular regulatory networks. In most cases, there are no experimental data on the reaction rates of involved proteins. So the criterion when choosing parameter values is to describe the normal behavior of the whole system with approximately correct proportion between self-renewing, differentiating and apoptotic cells and the correct response of the system to stimulation by the hormones. The set of parameters satisfying these conditions may not be unique.

Here, parameter values are chosen such that the system has three steady states, and values are given in Table 1. In this case, the system exhibits bistability, with one unstable steady state, and two stable ones. This is illustrated in Figure 2.1, where points A and C are stable nodes, and point B is a saddle. Point A corresponds to high levels of activated Fas and low levels of activated Erk, whereas point C corresponds to low levels of activated Fas and high levels of activated Erk. Therefore when the concentration of (Erk,Fas) converges to A , the cell will die by apoptosis or differentiate, yet when the concentration of (Erk,Fas) converges to C , the cell will divide into two

identical daughter cells. More detailed analysis of this system of equations is presented in [11, 15].

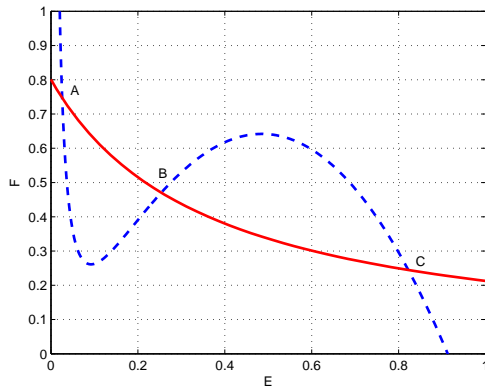


FIG. 2.1. Phase portrait of system (2.5).

The intracellular regulation scheme described above concerns erythroid progenitors but not reticulocytes. Indeed, reticulocytes are differentiated cells that only proliferate and eventually eject their nuclei to become erythrocytes. However, reticulocytes produce Fas-ligand, which influences intracellular regulation of erythroid progenitors (the second equation in system (2.5) depends on Fas-ligand). For large values of Fas-ligand, trajectories of the system move towards greater values of F_i and to smaller values of E_i .

Let us stress out the conditions that determine erythroid progenitor fate. If during the cell cycle, the value of Erk becomes greater than a given critical value E_c , then the cell will self-renew: at the end of the cell cycle, it will divide and give rise to two daughter cells that are identical to the mother cell. During the same cell cycle, if the value of Fas becomes greater than a given critical value, F_c , then the cell will die by apoptosis. If none of these conditions are satisfied, then the cell will differentiate: when the cell cycle is finished, the cell divides and gives birth to two more mature cells, that are reticulocytes.

Once produced, a reticulocyte remains in the computational domain during one more cell cycle, during which it produces Fas-ligand, and then becomes a mature erythrocyte. At the end of their development, reticulocytes leave the bone marrow and enter the bloodstream as red blood cells. In the framework of our model, reticulocytes that have performed one cell cycle are removed from the computational domain.

We note finally that the duration of cell cycle for each cell is considered as a random variable with the uniform distribution in the interval $[T_c - \tau, T_c + \tau]$, where T_c is the mean cell cycle time and $\tau < T_c$ is a parameter accounting for variability of the cell cycle duration.

Extracellular regulation. Reticulocytes interact with erythroid progenitors by direct cell-cell contact. As previously mentioned, reticulocytes produce Fas-ligand, which binds to the Fas receptors located on the surface of erythroid progenitors, and stimulates apoptosis and differentiation. For convenience, Fas-ligand distribution in space is modelled with a reaction-diffusion equation,

$$(2.6) \quad \frac{\partial FasL}{\partial t} = D\Delta FasL + W - \sigma FasL,$$

where W is a source term proportional to the concentration of reticulocytes. If the diffusion coefficient is sufficiently small, Fas-ligand is concentrated in a small vicinity of reticulocytes. An estimate of the diffusion coefficient is given in [8]. In this case, Fas-ligand influences erythroid progenitors when they are sufficiently close to reticulocytes. This is the situation illustrated in this work.

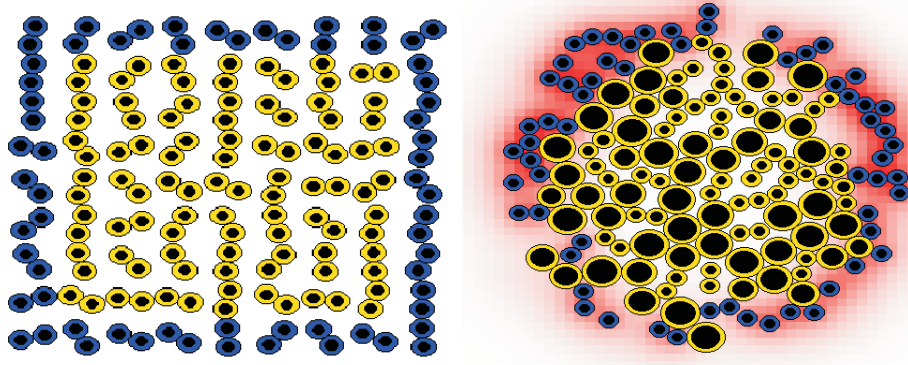


FIG. 2.2. *Erythroblastic island: yellow cells in the center are erythroid progenitors, blue cells at the border are reticulocytes producing Fas-ligand (in red). The initial cell distribution is on the left panel, the typical configuration of the island after several cell cycles on the right panel.*

Figure 2.2 shows the evolution of an erythroblastic island, by considering only the influence of Fas-ligand (Epo concentration is fixed). The left panel represents the initial cell configuration with immature progenitors inside and more mature reticulocytes outside. The right panel shows the typical structure of the island several cell cycles later. Extracellular Fas-ligand concentration (shown in red color) is higher near reticulocytes. Fas-ligand stimulates intracellular production of Fas in erythroid progenitors: if an erythroid progenitor is close to a reticulocyte, then the extracellular concentration of Fas-ligand and, as a consequence, the intracellular concentration of Fas in the progenitor can be sufficiently high to induce its apoptosis. Progenitors located at some distance from reticulocytes can have an intermediate value of intracellular Fas. Such cells will preferentially differentiate into reticulocytes. Finally, progenitors located far from reticulocytes mostly self-renew.

It is clear from this description that the evolution of an erythroblastic island depends on its size. In a small island, less progenitors will self-renew and the island will disappear after several cell cycles. On the contrary, if the island is sufficiently large, more progenitors will self-renew and the island will grow. Typical examples (obtained through 40 simulations, with the quartiles represented by dashed lines) are shown in Figure 2.3 where green lines represent the number of progenitors, blue lines the number of reticulocytes. Figure 2.3a shows normal erythropoiesis with approximately stable number of progenitors. Figure 2.3b shows excessive self-renewal that may correspond to leukemia, which will be discussed in the following subsection.

It is interesting to note that even when the average number of progenitors across 40 simulations is approximately constant (Figure 2.3a), in some simulations cell population vanishes or grows infinitely. It can be expected that the island should be stabilized by Epo, whose production depends on the number of erythrocytes in blood. Erythropoietin decreases apoptosis through inhibition of caspases. In the model of intracellular regulation, this corresponds to increasing the critical value of Fas, denoted

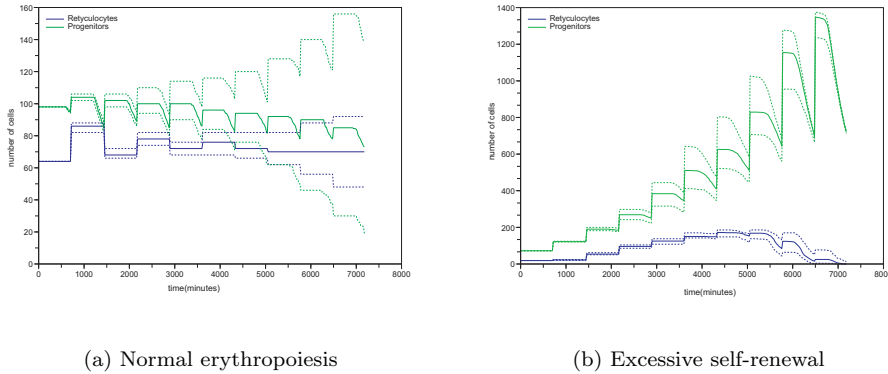


FIG. 2.3. *Evolution of cell populations. Green line represents the number of progenitors. Blue line represents the number of reticulocytes. Solid lines indicate means and dotted lines the quartiles over 40 simulations.*

by F_c . Figure 2.4 shows the proportions of apoptotic, differentiating and self-renewing cells for different critical values of Fas. If this critical value increases, then the proportion of apoptotic cells decreases while the proportion of differentiating cells increases and their sum remains approximately constant. At the same time, the proportion of self-renewing cells decreases.

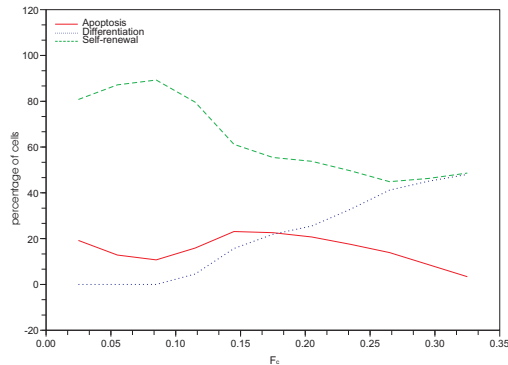


FIG. 2.4. *Proportion of self-renewing (green), apoptotic (red) and differentiating (blue) cells for different critical values of Fas. Each point is the average value of 40 simulations.*

2.3. Ara-C kinetics. One of the most common and effective chemotherapeutic agents against acute myelogenous leukemia is cytosine arabinoside, also known as Ara-C. Our aim is to apply a treatment with Ara-C to a population of erythroid cells formed with normal and leukemic cells, using the hybrid model described in the previous paragraphs. We describe hereafter the pharmacokinetics and pharmacodynamics of Ara-C.

Once introduced intravenously in the organism, Ara-C penetrates cell membranes

by a mechanism based essentially on the human equivibrative nucleoside transporter-1 (hENT1), which can be considered as facilitated diffusion. This process does not need energy and goes in accordance with the gradient of concentration up to the equilibrium between the intracellular and extracellular concentrations of Ara-C.

Inside cells, part of Ara-C is phosphorylated into its active form, Ara-CTP, which arrests DNA synthesis and induces cell apoptosis. The rest, about 70%, of Ara-C is deaminated into its inactive form Ara-U. Ara-CTP, in turn, can be dephosphorylated back to Ara-C. We take into account these three reactions in order to model the intracellular kinetics of Ara-C and Ara-CTP (see Figure 1.2). We describe them by Michaelis-Menten mechanisms. The phosphorylation rate of Ara-C, denoted by r_p , is given by

$$r_p = \frac{V_k}{1 + \frac{K_m K_a}{w_i}},$$

where w_i is the Ara-C concentration inside the i -th cell, K_m is the Michaelis-Menten constant, K_a accounts for additional effects and V_k is the kinase activity.

Denote by w_i^a the Ara-CTP concentration in the i -th cell. Ara-CTP dephosphorylation rate, r_{dp} , is given by

$$r_{dp} = \frac{V_{dp}}{1 + \frac{\alpha^1 K_{dp}}{w_i^a}},$$

with V_{dp} the enzyme activity, α^1 accounts for additional effects, K_{dp} is the Michaelis-Menten constant.

Finally, deamination of Ara-C follows similar kinetics, with r_{da} the rate of deamination,

$$r_{da} = \frac{V_{da}}{1 + \frac{K_{da}}{w_i}},$$

and V_{da} is the enzyme activity and K_{da} the Michaelis-Menten constant.

The kinetics of intracellular Ara-C concentration in the i -th cell is then described by the following equation,

$$(2.7) \quad \frac{dw_i}{dt} = k_1(w - w_i(t)) - k_2 w_i(t) - (r_p - r_{dp} + r_{da}),$$

where w is the extracellular concentration of Ara-C in the bone marrow, supposed to be uniformly distributed in the bone marrow, yet varying with time ($w = w(t)$); In fact, cells are infused in Ara-C for two hours, then Ara-C is removed (or washed out). The constant k_1 is related to the rate of membrane penetration, k_2 accounts for degradation of Ara-C. There is no extracellular degradation of Ara-C.

The balance equation for the intracellular concentration of Ara-CTP is

$$(2.8) \quad \frac{dw_i^a}{dt} = k_\alpha(r_p - r_{dp}).$$

Ara-CTP exerts a cytotoxic effect on all dividing cells, arresting DNA synthesis and inducing apoptosis. Evolution of the quantity p_i of Ara-CTP in DNA is described with the following equation,

$$(2.9) \quad \frac{dp_i}{dt} = k_\beta w_i^a,$$

with k_β the rate of conversion of Ara-CTP. When p_i overcomes a critical value p_{cr} , then it is assumed the cell dies by apoptosis. Above equations and parameters for Ara-C metabolism are derived from Morrison et al. [25].

TABLE 2.1
Parameter values (N.U. means “no unit”, min is for “minute”)

Parameter		Value	Unit
<i>Force between two cells</i>			
H_1	Cell compressible part	1.75	μm
K	Positive parameter	1000	
<i>Intracellular regulation</i>			
α	Epo-dependent Erk activation rate	0.0003472	min^{-1}
k	Erk self-activation power	2	N.U.
β	Erk self-activation rate	0.05	min^{-1}
a	Erk degradation rate	0.004	min^{-1}
b	Erk inhibition constant	0.0138	min^{-1}
γ_0	Constant Fas activation rate	0	min^{-1}
k_γ	Fas-ligand-dependent Fas activation rate	0.0002	min^{-1}
c	Fas inhibition rate	0.00138	min^{-1}
d	Fas degradation rate	0.0001	min^{-1}
E_c	Erk critical level	0.4	N.U.
F_c	Fas critical level	0.2	N.U.
<i>Extracellular regulation</i>			
D	Fas-ligand diffusion coefficient	$1e^{-5}$	$\mu\text{m}^2.\text{min}^{-1}$
σ	Fas-ligand degradation rate	0.02	min^{-1}
W	Source term	0.0001	min^{-1}
<i>Metabolism of AraC</i>			
k_1	Membrane penetration rate	0.0116	min^{-1}
k_2	Ara-C degradation rate	0	min^{-1}
V_k	Kinase activity	1.28	$\mu\text{M}.\text{min}^{-1}$
K_m	Michaelis-Menten constant	27.0	μM
K_a	Additional effects constant	16	N.U.
V_{dp}	Enzyme activity	300	$\mu\text{M}.\text{min}^{-1}$
K_{dp}	Michaelis-Menten constant	900	μM
α^1	Additional effects constant	44.4	N.U.
V_{da}	Enzyme activity	16.45	$\mu\text{M}.\text{min}^{-1}$
K_{da}	Michaelis-Menten constant	1011.7	μM
k_α	Metabolism constant	0.8	N.U.
k_β	Ara-CTP conversion rate	1	N.U.
p_{cr}	Critical value of Ara-CTP in DNA	40	μM
<i>Circadian rhythm</i>			
c_x	Constant characterizing the circadian clock	$\in [0, 1]$	N.U.
θ	Phase shift	5	hours

2.4. Organization and duration of cell cycle. Generally speaking, cell cycle proceeds through G_1 , S , G_2 and M phases, and cells can also enter a quiescent state, named G_0 phase. Duration of this resting phase and transition to cell cycle are

controlled by various intracellular and extracellular mechanisms. Erythroid progenitors are believed to be basically in the cell cycle with a short or inexistent G_0 phase. Taking this into account, we consider a single G_0/G_1 phase, whose duration varies randomly in some given interval. Denote by $G_0/G_{1_{min}}$ and $G_0/G_{1_{max}}$ the minimal and maximal values, respectively, of the G_0/G_1 phase and assume that duration of this phase is a random variable with a uniform distribution between them.

This assumption corresponds to biological observations [28]. From the modelling point of view, variation in the G_0/G_1 phase duration, and consequently in cell cycle duration, allows cells to desynchronize. This is essential for correct modelling of Ara-C pharmacodynamics and treatment of leukemia. Figure 2.5 shows two simulations of the complete model with $G_0/G_{1_{min}} = 0$ and different values of $G_0/G_{1_{max}}$. In the case $G_0/G_{1_{max}} = 7$ hours, after approximately 7 cell cycles the proportion of cells in the S phase becomes almost constant. For shorter cell cycle durations, $G_0/G_{1_{max}} = 3$ hours, the proportion of cells in S phase is strongly oscillating, even after several cell cycles, so cells are mainly not desynchronized.

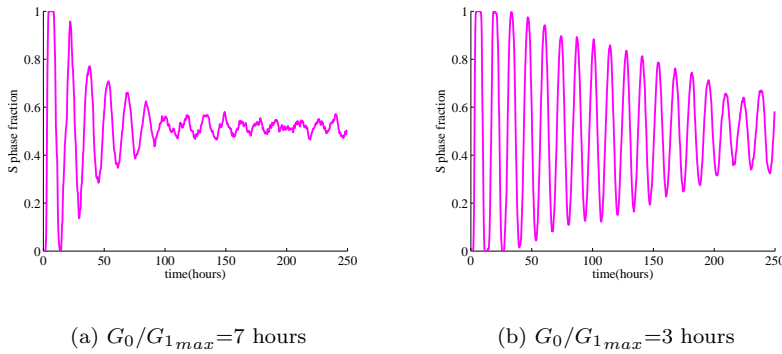


FIG. 2.5. Proportion of cells in phase S , with $G_0/G_{1_{min}} = 0$.

2.5. Circadian rhythm in the cell cycle. Circadian rhythms can influence proportion of cells in the S phase. Their ratio with the total number of cells is not constant during the day. Therefore efficiency of drug administration can also depend on time [2]. In order to describe circadian rhythms, we introduce a variable x that describes the concentration of cyclin-D1, a circadian clock control protein, whose activity is required for cell cycle G_1/S transition. Let us assume that this value x is in the interval $[0, x_{max}]$, where x_{max} is a threshold value at which the cell enters S phase. At the time of cell division (cell birth) t_0 we prescribe $x(t_0) = x_0$, where the initial concentration of the protein x_0 is a random variable uniformly distributed in $[0, x_{max}]$.

Since the corresponding gene is controlled by the circadian rhythm, the cyclin-D1 protein concentration is determined by the time of the day. This is reflected in the following equation for the concentration of the protein,

$$(2.10) \quad \frac{dx}{dt} = 1 + c_x \sin(f(t - \theta)),$$

where $f = \frac{2\pi}{24}$, $c_x \in [0, 1]$ and θ is a phase shift chosen to reproduce circadian timing

of cell division in humans. Solution to (2.10) is given by the formula:

$$(2.11) \quad x(t) = x_0 + (t - t_0) + \frac{c_x}{f} \cos(f(t_0 - \theta)) - \frac{c_x}{f} \cos(f(t - \theta)),$$

Assume that $\tau = t - t_0$, where τ is the time spent in phase G_0/G_1 . Then we have

$$(2.12) \quad x(t_0 + \tau) = x_0 + \tau + \frac{c_x}{f} \cos(f(t_0 - \theta)) - \frac{c_x}{f} \cos(f(t_0 + \tau - \theta)),$$

If $x(t_0 + \tau) = x_{max}$, the concentration of the protein attains the G_1/S transition threshold value, and the cell enters the S phase. That way, G_0/G_1 phase duration τ is a random variable distributed in $[0, \tau_{max}]$, and τ is also modulated by the circadian clock. The maximal value of τ , τ_{max} , corresponds to the threshold x_{max} .

3. Results. In this section, we numerically simulate the hybrid model presented in Section 2.1, considering a population formed with normal or leukemic erythroid progenitors, and reticulocytes. Each progenitor cell is characterized by proteins concentrations evolving according to the properties presented in Section 2.2. In particular, cell fate of the i -th cell depends on the values of Erk and Fas concentrations, E_i and F_i : during a cell cycle, if Erk concentration reaches the critical value E_c then the cell will self-renew at the end of its cycle, if Fas concentration reaches its critical value F_c then the cell will die by apoptosis immediately, in the other case the cell will differentiate in reticulocytes. These latter cells play an active role in progenitor fate, by releasing Fas-ligand, which induces Fas concentration increase. Since the feedback control by Fas-ligand is the only one considered for the simulations of this model, cell fate depends exclusively on the distance between progenitors and reticulocytes (Fas-ligand diffuses in the domain, according to equation (2.6)).

First, we focus on the effect of a treatment (by injecting Ara-C, see Section 2.3) on cell proliferation and survival. We investigate the respective influences of the period of the treatment and the starting hour of the treatment with respect to the cell cycle duration. Actually, cell cycle durations are randomly distributed between two critical values (see Section 2.4), $G_0/G_{1_{min}}$ and $G_0/G_{1_{max}}$. Except for one example in Subsection 3.1, $G_0/G_{1_{min}} = 5$ hours and $G_0/G_{1_{max}} = 31$ hours.

Second, we simulate the effect of a chronomodulated treatment with Ara-C (see Section 2.5), in the situation where normal cells are subject to circadian rhythm (so $c_x = 0.5$ in (2.12)) whereas leukemic cells do not exhibit circadian rhythm dependency (hence, $c_x = 0$). Effects of the treatment are analyzed with respect to the time of treatment compared to the cell position in the cell cycle.

In both cases, the cell population is first simulated from an initial state until it reaches a satisfactory state, in which the erythroblastic islands is large enough and cells are well mixed (see Figure 2.2, right panel). Then numerical experiments start with Ara-C treatment. Consequently, time $t = 0$ in every picture must be understood as the time of the beginning of the numerical experiments and not as the beginning of erythroblastic island simulations.

3.1. Treatment protocol depending on cell cycle duration. We first consider a population of erythroid cells, composed with two types of cells: immature progenitors and reticulocytes, and using the hybrid model previously presented we perform a simulation of treatment by Ara-C injections (all cells in the domain are in contact with Ara-C, which does not degrade in the extracellular medium and is uniformly distributed in the domain, for 2 consecutive hours, then Ara-C is removed).

We investigate the relevance of taking cell cycle durations into account when treating with Ara-C.

Because the cytotoxic effect of Ara-C is related to DNA synthesis, time periodic drug administration killing cells in the S phase will influence consecutive cell generations. This can result in so-called resonance effect, first discussed in [16]. If the periodicity of treatments is close to the duration of the cell cycle or to its multiples, then cells that are not in the S phase during one administration will be unlikely to be in S phase at subsequent administrations. These cells will divide resulting in growth of cell population.

Numerical simulations of erythroid cell populations with an average duration of cell cycle equal to 31 hours and with different protocols of drug administration are shown in Figure 3.1. For the same total amount of injected Ara-C, the cell population can either disappear (Figure 3.1a) or grow (Figure 3.1b,c) if the period of drug injections is far or close to multiples of cell cycle duration.

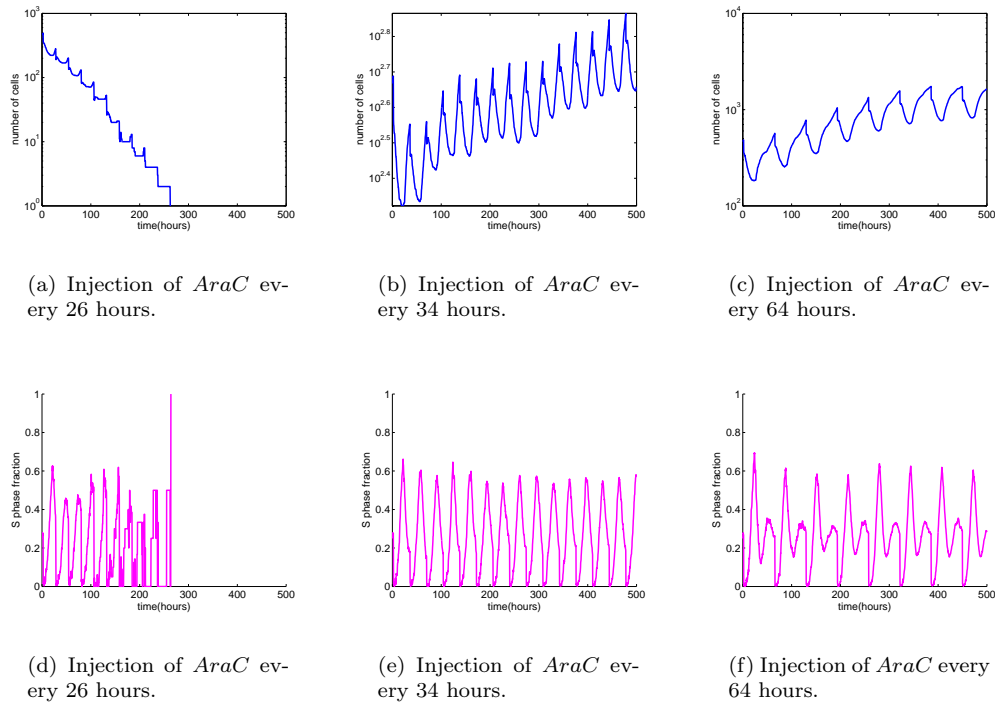


FIG. 3.1. Evolution of erythroid cell population under treatment with Ara-C. Upper row: Number of progenitors in cell population. Lower row: Proportion of cells in S phase.

Cell population growth or decay is approximately exponential with periodic modulations due to the action of the treatment. Considering that cell population evolves proportionally to $e^{\lambda t}$, where λ is the growth exponent (either positive or negative), Figure 3.2 shows the value λ as a function of the period of treatment T for two different values of cell cycle duration. In the first case, the duration of the G_0/G_1 phase is random and uniformly distributed between $G_0/G_{1_{min}} = 5$ hours and $G_0/G_{1_{max}} = 31$ hours, the duration of the S phase is 10 hours and of G_2/M phases is 3 hours. Hence the average cell cycle duration T_c is 31 hours, and the variation ± 13 hours. In the

second case, T_c is 33.5 hours and the variation is $\pm 15,5$ hours. The function $\lambda(T)$ in the left panel has a peak at approximately 33 hours close to the average cell cycle duration. The second maximum is much wider but corresponds to approximately twice the duration of cell cycle. Similar results are presented in the right picture for another value of cell cycle duration. The first peak is at 38 hours, close to the average cell cycle.

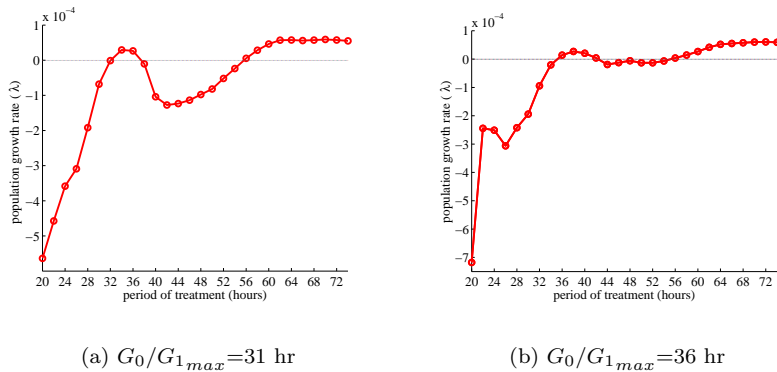


FIG. 3.2. Growth exponent λ as a function of the treatment period T .

These first results of numerical simulations with hybrid models confirm that the search for an optimal treatment period is relevant within the context of erythroid cell populations. Depending on the period of the treatment with Ara-C, compared to the average cell cycle duration, the progenitor population can either grow or decay.

3.2. Optimal administration of Ara-C related to circadian rhythm.

Most cytotoxic drugs used in chemotherapy are not specific to malignant cells. They affect both normal and leukemic cells. However, in leukemia, downregulation of circadian clock genes is often observed. Malignant cells can even escape circadian control. Thus, treatment protocol should take into account this difference in normal and leukemic cells in order to reduce undesirable effects of chemotherapy and to increase its efficacy.

Let us consider two different populations of cells. The first one consists of erythroid progenitors that follow circadian rhythms with a certain synchronization in their DNA synthesis activity. This population is characterized by a coefficient $c_x = 0.5$ in equation (2.12). The second population consists of leukemic cells that do not obey the circadian control. Consequently, this population is characterized by a coefficient $c_x = 0$ in (2.12).

We first simulate cell population dynamics, considering a duration of $S/G_2/M$ phases equal to 13 hours and a duration of G_0/G_1 phase varying according to circadian rhythms between $G_0/G_{1_{min}} = 5$ and $G_0/G_{1_{max}} = 31$ hours. The fraction of erythroid progenitors in the S phase is then maximal around 1 p.m. and minimal between midnight and 1 a.m. (see Figure 3.3), in agreement with clinical observations [31].

Second, we simulate the chronomodulated administration protocol of Ara-C with intervals ranging from 8 hours to 65 hours. The total dose of Ara-C administrated is conserved, and day time delivery t_0 of the treatment is taken every hour, between 1:00 and 24:00 (Figure 3.4a). Outcomes of the model, chosen here to be measurements of

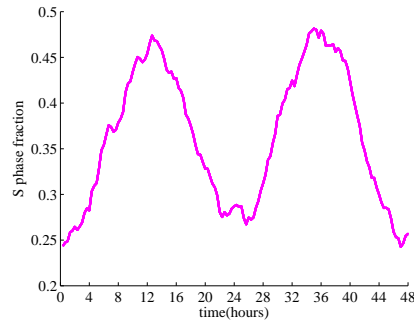


FIG. 3.3. *Fraction of cells in the S phase.*

the growth rate λ , are then functions of only the period of treatment and the initial time t_0 .

As expected, the most important variations of growth rate as a function of t_0 together with the best values of the growth rate are observed for periods of treatment equal to 24 hours (Figure 3.4b) and 48 hours (Figure 3.4c). Apart from these periods, the delivery time does not appear to modify the outcome of the simulations (Figure 3.4a). Low values (black) indicate bad treatment outcomes and high values (white) indicate good treatment. It is noticeable, however, that if period of treatment is slightly larger (or less, resp.) than 24 hours or 48 hours, then the time of first delivery t_0 should be advanced (or delayed, resp.) to produce an equivalent outcome. This dependence is more brightly expressed at 48 hours (Figure 3.4c) [3].

The timing of a 24-periodic treatment (Figure 3.5a) and 48-periodic treatment (Figure 3.5b) affects the treatment toxicity. If Ara-C is administrated at 1 a.m., when the lowest fraction of cells are in the *S*-phase (see Figure 3.3), the total cell number decreases slowly (upper curve). If Ara-C is introduced at 1 p.m., when more cells are in the *S*-phase (Figure 3.3), then the cell population decreases faster (lower curve). With the periodicity of treatment 48 hours and the time of first delivery 1 a.m., the population of cells decreases more slowly than with periodicity of treatment 24 hours and the same time of first delivery (Figure 3.4). As mentioned above, it is the population of normal cells that do obey the circadian control. So, it can be deduced, that a treatment with 48 hours period administrated at the better time t_0 gives less toxicity, than the same administration with a 24-periodic treatment. Moreover, the worst treatment is at 1 p.m. with a 48-periodic treatment, and the best time to treat is at 1 a.m.

This population is characterized by a coefficient $c_x = 0.5$ in equation (2.12). The second population consists of leukemic cells that do not obey the circadian control. Consequently, this population is characterized by a coefficient $c_x = 0$ in

Next, we compare the response to treatment of normal cells, which follow circadian rhythm, and of leukemic cells, which escape it. Populations of normal (green line) and leukemic cells (red line), subjected to 48 h interval treatment at different times t_0 , are presented in Figure 3.6. The population of normal cells remains approximately constant while the population of leukemic cells dies when t_0 is 1 a.m. (Figure 3.6a). On the contrary, the population of normal cells dies even faster than the population of leukemic cells if first time delivery is at 1 p.m. when the toxicity of the drug is extremely large (Figure 3.6b).

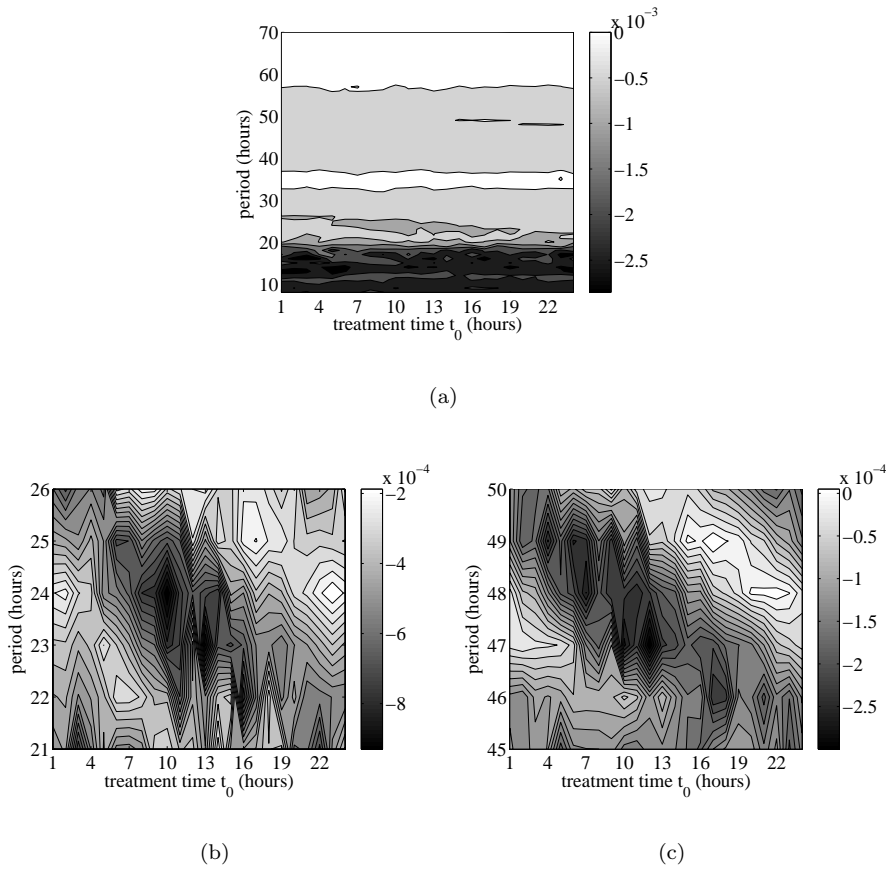


FIG. 3.4. Treatment outcomes λ as a function of the first day delivery time t_0 and the period T between two consecutive drug administrations. Panel (a) represents the growth exponent λ for t_0 ranging from 1 a.m. to midnight, and T ranging between 8 and 65 hours. Panel (b) is a focus of Panel (a) around the period $T = 24$ hours, and Panel (c) a focus around the period $T = 48$ hours.

4. Discussion. We considered a hybrid model, formed with discrete cell population dynamics coupled to continuous intracellular and extracellular regulation of cell fate, in order to investigate effects of a leukemia treatment (in our case, erythroleukemia) and, more particularly, the relevance of a chronotherapy. The resulting model is strongly complex, yet it correctly describes the current knowledge on erythropoiesis, including feedback controls by hormones (erythropoietin, for instance) as well as cell-cell interactions in the bone marrow. It allowed considering a treatment of leukemia with Ara-C, and showed how relevant it is to correctly choose the time of administration, together with the period of treatment, in order to avoid killing more normal cells than leukemic cells. It can be noted that leukemic cells are practically irresponsive to intracellular and extracellular regulation. From the modelling point of view developed in this work, this means that the regulation described in Section 2.2 does not influence leukemic cells. Hence the analysis of pharmacokinetics and pharmacodynamics of leukemic cells, which was one of the main objectives of this work, is applicable not only for erythroleukemia but also for all types of acute leukemia.

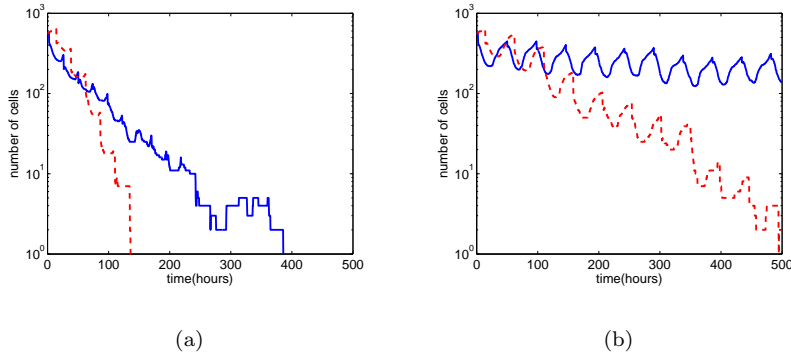


FIG. 3.5. Evolution in time of the total number of cells with Ara-C administrated at 1 a.m. (upper curve) or at 1 p.m. (lower curve). Left: Interval between administrations is 24 hours. Right: Interval between administrations is 48 hours.

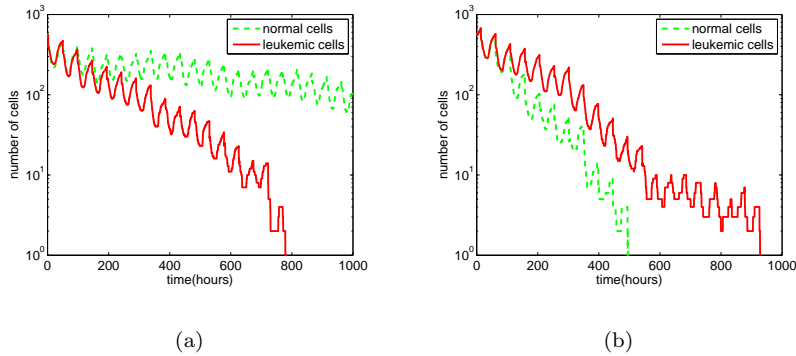


FIG. 3.6. Evolution in time of the total number of normal cells (green line) and leukemic cells (red line) with Ara-C administrated every 48 hours at 1 a.m. (left) and at 1 p.m. (right).

On the other hand however, when analyzing the influence of chemotherapy on normal cells, we need to take into account their regulation.

The subsequent analysis of a chronomodulated treatment showed that it was possible to have efficacy of the treatment (kill leukemic cells) and reduced cytotoxicity for normal cells. It is necessary to consider variations in cell cycle durations of normal cells and to determine the treatment protocol according to the distribution of cells in S phase.

In this work, a simplified erythropoiesis model was used, by not considering the feedback control by Epo on progenitor cell proliferation and apoptosis. Only the feedback control mediated by reticulocytes, through Fas-ligand release, that induces progenitor apoptosis was taken into account. This was sufficient to induce cell differentiation, however erythroblastic islands are not very stable: after at most 10 cell cycles cell populations either decay and finally disappear, or grow indefinitely. In order to simulate cell populations over several generations, erythroblastic islands must be stable, and a way to get such a stability is to better describe the nature of the

islands, by considering that cells are distributed around a macrophage. Simulations (not shown here) indicate that the macrophage stabilizes the erythroblastic islands, through the feedback of mature erythrocytes in blood on Epo levels. This question will be discussed in a subsequent work [4].

Regarding the chronomodulated treatment, it should be noted that resonance is observed in the simplified model while the real *in vivo* situation can be much more complex. First of all, cell lineages with different durations of cell cycle can coexist. Moreover, treatment itself can change duration of cell cycle, delaying the process of DNA duplication [25]. To our knowledge, resonance effect has not yet been observed *in vivo*.

One would have noticed that, in order to use hybrid models and methods in cancer modelling and treatment, development of several submodels are required. First, rather sophisticated computational tools or frameworks must be developed, including: a) cells as individual objects that can divide, move, change their type (differentiation), die (disappear from the computational domain), interact with each other and with the surrounding medium mechanically and chemically; b) intracellular regulatory networks described, for instance, by ordinary differential equations; c) extracellular biochemical species, whose evolution can be described by partial differential equations. Second, detailed knowledge of intracellular and extracellular regulations are needed. Extracellular regulation can include local regulation due to cell communication in the tissue or global feedback through hormones or other signaling molecules produced in other organs. Intracellular and extracellular regulations can influence each other in a complex way. Third, pharmacokinetics of medical treatment, treatment protocol, some particular biological or medical aspects of the problem under consideration, like for instance resonance and chronotherapy in this work, must be modeled.

Developing and putting together these three parts takes years of work of rather big inter-disciplinary groups. There are two major obstacles in the development of this approach: values of parameters and availability of clinical data. Some values of parameters can be found in the literature. In many cases, however, reaction rates in intracellular protein cascades are completely unknown. Moreover, a “complete” reaction scheme can contain hundreds of interacting proteins, genes, and other factors. So we need to simplify the model preserving its most essential features. The values of parameters should be adjusted in order to obtain biologically realistic functioning of the system: correct proportion of each cell type, correct response to various control parameters, and so on. The uncertainty of the choice of parameters will nevertheless often remain.

Beyond the question of access to the existing clinical data, which is not straightforward, the main difficulty when trying to compare simulations to existing data, and possibly suggest an improved treatment protocol, is related to the extreme complexity of *in vivo* processes. Intra-specific variation, which has a crucial importance from the evolutionary point of view, complicates even more treatment optimization for individual patients. In spite of these difficulties, we can expect that hybrid models will be used in clinical practice.

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