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Studying Non-Linear Phenomena of Tumour Cell Populations under Chemotherapeutic Drug Influence[1]

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Abstract: Biological systems are characterized by their potential for dynamic adaptation. Such systems, whose properties depend on their initial conditions and response over time, are expected to manifest non-linear behaviour. In a previous work we examined the oscillatory pattern exhibited by leukemic cells under *in vitro* growth conditions, where the system was simulating the dynamics of growth with disease progression. Our question in a previous study evolved around the nature of the dynamics of a cell population that grows, or even struggles to grow, under treatment with chemotherapeutic agents. We mentioned several tools that could become useful in answering that question, as for example the *in vitro* models which provide information over the spatio-temporal nature of such dynamics, but *in vivo* models could prove useful too.

In the present work we have studied the non-linear effects that arise from cell population dynamics during chemotherapy. The study was performed not only in the sense of cell populations *per se* but also as an attempt of identifying sub-populations of cells, such as apoptotic cells and cells distributed within the cell cycle. The temporal transition from one state to the next was revealed to follow non-linear dynamics. We have managed to approximate the non-linear factor that influences these temporal space transitions. Such approaches could become very useful in understanding the nature of cell proliferation and the role that certain chemotherapeutic drugs play in cell growth, with emphasis given on the underlying drug resistance and cell differentiation mechanisms. Further on, we have attempted to approach this problem by using experimental data using the case of glucocorticoids. Glucocorticoids are considered to be indispensable agents in the treatment of hematologic malignancies. A critical established glucocorticoid action is the apoptotic effect that they exert on leukemic cells. However, little is known about the molecular response of malignant cells on glucocorticoid exposure. Even less is known about the cell proliferation dynamics governing leukemic cells under glucocorticoid influence. Dynamic parameters of the cell population state, like growth rate or its time derivative, are largely overlooked in cell population studies. In the present work a quantitative mathematical and modeling approach is endeavored regarding growth and metabolic dynamics. Cell populations and metabolic factors, such

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as glucose, lactate and lactate dehydrogenase (LDH) are measured. Growth and metabolic features are assumed to be of nonlinear nature. A model-based prediction of glucocorticoid effects is derived by applying a non-linear fitting approximation to the measured parameters.
To the best of our knowledge there are not many studies dealing with this topic, which makes it even more interesting.

Keywords: Proliferation, oscillations, non-linearity, CCRF-CEM, glucocorticoids.

1 Introduction

Population dynamics have been the subject of study among various groups. It has already been shown that even cells that grow under normal conditions can manifest proliferation dynamics of non-linear nature [1, 2]. In addition, other groups have demonstrated that this non-linear behavior can also exist under the influence of drugs [3], or similarly, under the influence of environmental factors. Any new knowledge on the mechanisms underlying cell proliferation is of major importance, and even the smallest of indications towards a certain direction could enable us to further discover differences in the mechanisms distinguishing between health and disease. This issue is especially important in tumors, the incidence of which is approaching that of an epidemic. In the present study we focused on the dynamics that were revealed through an *in vitro* cell system, and particularly on the dynamics manifested under the influence of a certain type of chemotherapeutic drug, such as glucocorticoids. Glucocorticoids (GC) are among the most important alternatives in the treatment of leukemia. Resistance to glucocorticoids represents a crucial parameter in the prognosis of leukemia [4-6], whereas it has been shown that GC-resistant T-cell leukemia cells manifest a biphasic mechanism of action or imply an inherent resistance mechanism of action to glucocorticoids [7]. New questions arise regarding the nature of the dynamics of a cell population under the influence of a drug. If certain physical measures, such as proliferation, are observed on the phenotypic level, how are they translated on the molecular / genomic level? For example, if a cell population increases its rate of proliferation, does it mean that the genes required for this effect transcribe faster than usual? An interesting report by *Mar et al. (2009*) suggested that gene expression takes place in quanta, i.e. that it happens discretely and not continuously [8, 9]. Also, in two other reports it was suggested that gene expression follows oscillatory patterns, which makes things even more complicated with regards to the proliferation rate, be it growth acceleration or deceleration [10, 11]. This means that cells cannot simply transit from one state to another in terms of growth rate. Should the hypothesis of oscillatory modulation of gene expression, which implies non-linearity, stand correct, then a much more complicated regulatory pattern is required by a cell so as to change its state, as a function of environmental stimuli. The present work provides evidence supporting this view, with respect to glucocorticoids. The answer on whether cells possess inherent mechanisms inducing GC tolerance or whether they develop resistance as a response to treatment remains elusive. In other

words, do cells evolve to a certain phenotype or they already possess traits such as drug resistance?

The same applies for critical aspects of the metabolism of cancer cells and in particular, leukemic cells. Already in 1924, *Warburg et al.* observed that a shift occurred in tumors from oxidative phosphorylation to aerobic glycolysis, known as the *Warburg effect* [12]. It is known, that metabolites, or metabolic molecules, do not only participate in metabolic processes related solely with energy production and thermodynamical conservation of the cell, but also mediate numerous signal transduction related functions.

We did not give emphasis on the molecular profile of proliferating cells but rather on cell populations as they are measured during glucocorticoid treatment, in a spatio-temporal manner. Previous works have dealt with this issue, giving emphasis on the glucocorticoid receptor and the pharmacokinetics of glucocorticoids (methylprednisolone) [13, 14].

The present work uses numerical analysis methods along with fitting and modeling approximations in order to establish a mathematical model for the analysis and prediction of the effects of glucocorticoids on T-leukemic cells. Also, we attempted to demonstrate the non-linear nature of the present biological system using experimental data from both proliferation measures and metabolic factor measurements, complementary to the theoretical aspects. We have also, tried to measure and calculate physical constants, such as, growth and consumption rate and its time derivative (the analogues of velocity and acceleration) of the observed processes, if such exist. Overall, the significance of the present work relies on the effort to set up a mathematical framework for the prediction of glucocorticoid effects on leukemic cells and its connection to non-linear phenomena. To the best of our knowledge, there are no previous reports on modeling the effects of glucocorticoids on leukemic systems.

2 Materials and Methods

Cell Culture and Prednisolone Treatments

The CCRF-CEM (ALL) cell line was obtained from the European Collection of Cell Cultures (ECACC) and was used as the model cell line. The T-Lymphoblastic Leukemia CCRF-CEM cells were grown in RPMI-1640 medium supplemented with 2mM L-Glutamine and Streptomycin/Penicillin 100 U/ml (Gibco, Carlsbad, CA), 20% FBS (Gibco, Carlsbad, CA) at 37^{o}C, 5% CO_2 and ~100% humidity. Cells were allowed to grow to ~900-4l 33 43^3 cells/ul for CCRF-CEM. The following concentrations of prednisolone (Pharmacia, Boston, MA) were used: 0 M (control), 10nM, 100nM, 1uM, 10 M, 100uM and 700 M [7].

Cell Population Measurements

Cell population counts were determined with the use of a NIHON KOHDEN CellTaq- hematology analyzer. Cells were counted at the -24 h time point as well as at 0 h, 4 h, 24 h, 48 h, 72 h after having been let to grow under normal

conditions. For this purpose, 200 l of cell suspensions were obtained from each flask and counted directly with the analyzer [7].

Biochemical Measurements

Supernatants from the cell culture were taken every 24h and kept at -80^{o}C thereafter until further processing. In brief, 1ml of cell culture media was centrifuged at 1200 rpm for 10min and the supernatant was removed and kept for further processing. Samples were then measured with a Siemens biochemical analyzer *Advia 1800*. The factors measured were Glucose (mg/dlt), Lactic Acid (mg/dlt), Lactate Dehydrogenase (LDH, IU/lt) and Alkaline Phosphatase (ALP, IU/lt).

Flow Cytometric Measurements

Flow cytometry was performed on a *Beckman Coulter* flow cytometer *FlowCount XL*. Cytotoxicity measurements were performed as previously described [7]. All experiments were performed in triplicate. The reported data constitute the average of three independent experiments.

Data Analysis

Flow cytometry and cell cycle data (cell cycle data not shown) were analyzed with *WinMDI* software version 2.8 (*The Scripps Institute, Flow Cytometry Core Facility*) and *Cylchred* version 1.0.2 (*Cardiff University, Wales)* which is based on the algorithms proposed by Watson et al. and Ormerod et al [15-17]. Raw data from cytometric studies were pre-

environment (The Mathworks Inc.).

3 Mathematical Formulations

Generalized Cell Population Dynamics under Drug Influence

In order to establish a modeling approach to the phenomenon described above, we discriminated between different cell populations. That is, if at time t a cell population is considered to be N, then this is a mixture of cells in various stages. More specifically, we have discriminated between the cell cycle phases and cell death. The cell cycle is the path through which cells manifest proliferation. The identification of cells in specific cell cycle phases is of critical importance, since it will determine cellular proliferation, cessation or cell death. Also, in various systems the detection of cells at specific cell cycle points, denotes a mechanism of reaction to an environmental stimulus, as for example in the present case is the glucocorticoid. In Figure 1, we present the model diagrammatically.

The three phases of the cell cycle are represented. $G_{1,t}$, $G_{1,t+1}$, $G_{1,t+n}$ is the number of cells in G_1 phase at time t, $t+1$ and $t+n$ respectively, S_t, S_{t+1}, S_{t+n} is the number of cells in S phase at time t, $t+1$, $t+n$, respectively, $G_{2,t}$ is the number of cells in G_2 phase at time t, $t+1$, $t+n$, respectively and CD_t, CD_{t+1}, CD_{t+n} is the number of dead cells at time t, $t+1$, $t+n$, respectively. The arrows

connecting the different cell states, denote the possibilities that a cell has to transit from one state to another. So, for example, a cell in G_1 phase has three possibilities: to remain in the G_1 phase, to transit to the S phase or to become apoptotic, such as cell death (CD). This means that it is impossible for the cell to go from the G_1 phase to G_2 phase. A very important factor shown in Figure 1, is the $K_{factor,t}$, which denotes the rate of transition from one cell state to another. Hence, the factor k will take the following subscripts:

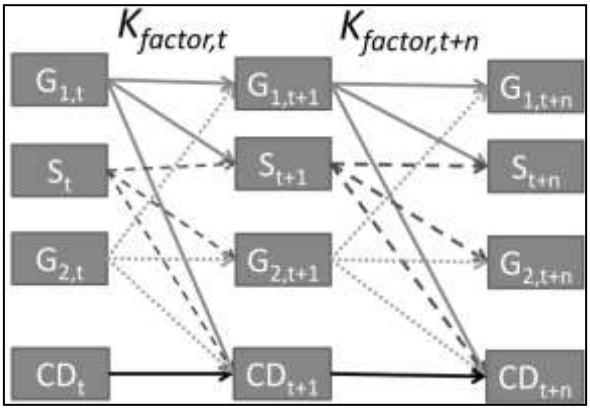

Fig. 1. A schematic representation of the model approach for cell population showing transitions between cell cycle phases and cell death.

$G_{1,t} \rightarrow G_{1,t+1}$: k_1, $G_{1,t} \rightarrow S_{t+1}$: k_2, $G_{1,t} \rightarrow CD_{t+1}$: k_3,
$S_t \rightarrow S_{t+1}$: k_4, $S_t \rightarrow G_{2,t+1}$: k_5, $S_t \rightarrow CD_{t+1}$: k_6
$G_{2,t} \rightarrow G_{2,t+1}$: k_7, $G_{2,t} \rightarrow G_{1,t+1}$: k_8, $G_{2,t} \rightarrow CD_{t+1}$: k_9
$CD_t \rightarrow CD_{t+1}$: k_{10}

The following equations describe the transitions from one state to the next:

$$N_{G_{1,t+1}} = N_{G_{1,t}} \cdot k_1 + N_{G_{2,t}} \cdot k_8$$

$$N_{S_{t+1}} = N_{S_t} \cdot k_4 + N_{G_{1,t}} \cdot k_2$$

$$N_{G_{2,t+1}} = N_{G_{2,t}} \cdot k_7 + N_{S_t} \cdot k_5$$

$$N_{CD_{t+1}} = N_{CD_t} + N_{G_{1,t}} \cdot k_3 + N_{G_{2,t}} \cdot k_9 + N_{S_t} \cdot k_6$$

Where, N denotes the respective cell population at time t. These equations could be formulated in more generalized form since each population at time $t+1$ consists of two other populations at time t. Hence, the generalized form would be: $N_{p_x,t+1} = N_{p_y,t} k_y + N_{p_z,t} k_z$

In other words, our model shows that the next state is defined by the previous one. Each cell subpopulation consists of parts of the other subpopulations.

These equations appear to be of linear form and are simple to solve. Yet, the factor *k* is a non-linear factor, which can be determined only experimentally. It is dependent upon environmental factors *f(environmental)*, such as nutrient availability and space, and in the present case is a function of glucocorticoid concentration $f(C_p)$. We have reported this previously, that cell populations defined experimentally, could be described with Fourier series, with respect to the transition factor *k* [12].

The generalized form of the series we have used for our approach was given by:

$$f(x,y) = a_0 + a_1 \cos(xy) + a_2 \sin(xy)$$

Hence, the factor *k* for each transition, meaning from one cell state to the next would be given by the following system of equations:

$$k_1 = a_{0,1} + a_{1,1} \cos(N_{G_{1,t}} \cdot N_{G_{1,t+1}}) + a_{2,1} \sin(N_{G_{1,t}} \cdot N_{G_{1,t+1}})$$

$$k_2 = a_{0,2} + a_{1,2} \cos(N_{G_{1,t}} \cdot N_{S_{t+1}}) + a_{2,2} \sin(N_{G_{1,t}} \cdot N_{S_{t+1}})$$

$$k_3 = a_{0,3} + a_{1,3} \cos(N_{G_{1,t}} \cdot N_{CD_{t+1}}) + a_{2,3} \sin(N_{G_{1,t}} \cdot N_{CD_{t+1}})$$

$$k_4 = a_{0,4} + a_{1,4} \cos(N_{S_t} \cdot N_{S_{t+1}}) + a_{2,4} \sin(N_{S_t} \cdot N_{S_{t+1}})$$

$$k_5 = a_{0,5} + a_{1,5} \cos(N_{S_t} \cdot N_{G_{2,t+1}}) + a_{2,5} \sin(N_{S_t} \cdot N_{G_{2,t+1}})$$

$$k_6 = a_{0,6} + a_{1,6} \cos(N_{S_t} \cdot N_{CD_{t+1}}) + a_{2,6} \sin(N_{S_t} \cdot N_{CD_{t+1}})$$

$$k_7 = a_{0,7} + a_{1,7} \cos(N_{G_{2,t}} \cdot N_{G_{2,t+1}}) + a_{2,7} \sin(N_{G_{2,t}} \cdot N_{G_{2,t+1}})$$

$$k_8 = a_{0,8} + a_{1,8} \cos(N_{G_{2,t}} \cdot N_{G_{1,t+1}}) + a_{2,8} \sin(N_{G_{2,t}} \cdot N_{G_{1,t+1}})$$

$$k_9 = a_{0,9} + a_{1,9} \cos(N_{G_{2,t}} \cdot N_{CD_{t+1}}) + a_{2,6} \sin(N_{G_{2,t}} \cdot N_{CD_{t+1}})$$

$$k_{10} = 1$$

We could write this system of equations in a more generalized form, which would be:

$$k = a_0 + a_1 \cos(N_{p_{y,z},t} N_{p_x,t+1}) + a_2 \sin(N_{p_{y,z},t} N_{p_x,t+1}) \text{ , [Eq. 1]}$$

Where *k* is the transition factor, $a_{0,1,2}$ are constants, $N_{p1,t}$ and $N_{p2,t+1}$ are the populations implicated in the transition at time *t* and *t+1* respectively.

Substituting the equation describing the generalized *k* with the equation of the generalized $N_{p,t+1}$ we obtain:

$$N_{p_{x,t+1}} = N_{p_{y,t}} \left[a_0 + a_1 \cos\left(N_{p_{y,t}} N_{p_{x,t+1}}\right) + a_2 \sin\left(N_{p_{y,t}} N_{p_{x,t+1}}\right) \right] + N_{p_{y,t}} \left[a_0 + a_1 \cos\left(N_{p_{z,t}} N_{p_{x,t+1}}\right) + a_2 \sin\left(N_{p_{z,t}} N_{p_{x,t+1}}\right) \right] \text{ , [Eq. 2]}$$

This equation describes the transition of a cell population from one state to the next but it cannot be solved analytically. Solutions can only be found numerically, since future populations (N_x) depend on the previous ones and on the fraction of other future cell populations ($N_{y,z}$).

TABLE I
SYMBOLS AND UNITS FOR VARIABLES

Symbol	Quantity	Units
N_t	Total cell population at time *t*	2 43^3
N_e	Cell population under an effect, *e* can take the following values: *v: viable* *n: necrotic* *a: apoptotic* *ea: early apoptotic* *ta: total apoptotic* *td: total cell death*	2 43^3
N_{G1}	Cell population in G_1 phase of the cell cycle	2 43^3
N_S	Cell population in S phase of the cell cycle	2 43^3
N_{G2}	Cell population in G_2 phase of the cell cycle	2 43^3
k	The factor by which total population proliferates from time *t* to time *t+1*.	
$K_{e,t}$	The factor by which cell population under a certain effect proliferates from time *t* to time *t+1*. *e* takes values as mentioned above in the same table	
C_G	Glucose concentration	mg/dlt
C_{LA}	Lactic Acid concentration	mg/dlt
C_{ALP}	Alkaline Phosphatase concentration	IU/lt
C_{LDH}	Lactate Dehydrogenase	IU/lt
k_m	The factor by which metabolic factors are produced or consumed. from time *t* to time *t+1*. *m* can take the following values: *G: Glucose* *LA: Lactic Acid* *ALP: Alkaline Phosphatase* *LDH: Lactate Dehydrogenase*	
u_m	Reaction rate (reaction kinetics)	M/sec

Metabolism Dynamics under Drug Influence

Besides the generalized population model, we also attempted to model the glucocorticoid effects, as far as metabolic factors are concerned. A mathematical model was set that enabled numerical solutions for the study of their effects. As described previously in the previous section, the model presumes that the fraction of cells linked to a certain phenotypic effect can be derived from the previous total cell population so, let $N_{e,t+1}$ be the cell population under a certain effect. This effect can be, for the present analysis, either viability or cell death. Therefore, the total population estimate under the impact of a given effect will be given by:

$N_{e,(t+1)} = k_{e,t} \cdot N_{e,t}$, (1) where $k_{e,t}$ is a generalized nonlinear coefficient of the effect e in the population $N_{e,t}$ at this instance.

At the same time, apart from cell proliferation, we have to account for metabolic factors that change over time and probably influence the course of proliferation. In the case of metabolic factors, the rate of change in concentration is defined as the rate of the respective reaction which is:

$u_m = \frac{dC_m}{dt}$ [**Eq. 3**]. However, in the present case two of the substances measured are glucose and lactic acid. It is known that glucose is transformed into two lactic acid molecules based on the reaction: $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$. This is due to the formation of two molecules of pyruvate from the anaerobic catabolism of glucose and the subsequent formation of two molecules of lactate in the cytosol. However, this reaction represents a lump reaction, namely one that represents the algebraic sum of many reactions. With many intermediates in between and therefore kinetic rules such as *Michaelis-Menten* or *Le Chatelier's/Van't Hoff* cannot be directly applied to these data. However, under the assumption that there is not significant biochemical cross-talk of these intermediates with other external metabolic pools, the lumping of the reactions to a single one, is plausible as is the case of lactate production through the catalysis of pyruvate. The substrates of this reaction were measured. LDH concentration can be accounted only from cells that were lysed and not from the total population. Although the LDH concentration can be numerically calculated, it would still not be a reliable numerical approximation. Therefore, we used the same principle as in the case of cell population. The concentration *C* of a metabolite or substance at time *t+1* can be written as:

$C_{m,(t+1)} = k_{m,t} \cdot C_{m,t}$, [**Eq. 4**]. Applying mass balance equations [18] for the metabolic pools with respect to time we have, $\frac{dC_m}{dt} = k_{m,t} \cdot C_{m,t}$ where $k_{m,t}$ is a generalized coefficient of the net effect observed in the pool $C_{m,t}$ at time *t*. This resembles a modification of the *Lotka-Voltera-Kolmogorov* equations which were initially used for the description of reaction dynamics and further expanded to population dynamics [19, 20]. The *Lotka-Voltera* functions were derived from the *Verhulst* logistic equation [21]. Though succinct this mathematical formulation introduces through the use of factor $k_{m,t}$, non-linearity. Coefficient $k_{m,t}$ bears a critical biological significance in the model. Presuming that the effects in this study are directly linked to glucocorticoid exposure, $k=f(C_p)$, where *p* stands for *prednisolone*, the glucocorticoid used in concentration. In order to approximate the values, i.e. numerically solve our

functions, we have used phase-space maps of the measured data. Symbols and definitions are given in Table I.

4 Results

The major challenge of computational and systems biology is to make contributions to the description of population and reaction dynamics [22]. This is applied to both systems under no external influences but also to systems under the influence of external stimuli, such as pharmacological interventions (as in the present case) or environmental stresses. In the case of the cell system studied in the present work, the most interesting observation was that the system was resistant to GCs and therefore our attempt was in fact to model dynamics of cellular growth and metabolism in resistant cases. Future research directions could point towards describing drug effects as a function of time or concentration and towards predicting the outcome of certain treatments or even towards improving the state of treatment in such a way, that it would be more effective. We suggest that the transition of the cell system that we have studied from one state to the next, follows complicated dynamics, manifesting in almost all cases oscillatory behaviour. The use of mathematical and modelling tools for the discovery of such mechanisms is a unique method for understanding complicated biological systems. Many research efforts are dedicated to the improvement of the existing or to the development of new pharmaceuticals. In Figure 2, experimental measurements are presented as an effort to calculate the rate of population change for the total population and data were fitted with Fourier series.

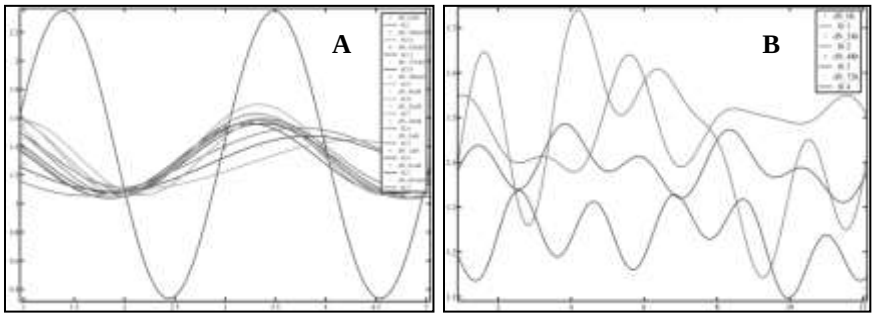

Fig. 2. Simulating the factor *k* in relation to time (**A**) and glucocorticoid concentration (**B**) showed that both could be fitted with Fourier series. In (**A**) the x-axis corresponds to experimental values from time point measurements of cell numbers, while each curve corresponds to the respective *k* factor of each glucocorticoid concentration. Similarly, in (**B**) the x-axis corresponds to the glucocorticoid concentrations and each curve corresponds to the time points measured.

Modelling approaches could assist in such efforts as they would provide with a more in-depth understanding of biological systems. The general idea is to be able to predict the future states of a system, based on the present ones. This is proved to be a difficult task, since biological systems follow nonlinear

behaviour and, unlike physical systems, there are only a few generalizations that can be formulated. In Figure 3, we have performed numerical approximations of the function (Eq. 2) in order to represent this schematically. The function appeared to give interesting dynamics, as it manifested a saddle point. Also, these phenomena were time dependent, as clearly seen on the experimental level. Thus, by differentiating with respect to time we could obtain a possible role of the temporal factor in this system. Similarly, we have made numerical approximations in order to design the dynamics of the first derivative for both variables, that is $N_{p,y}$ and $N_{p,z}$. The result is presented in Figure 4.

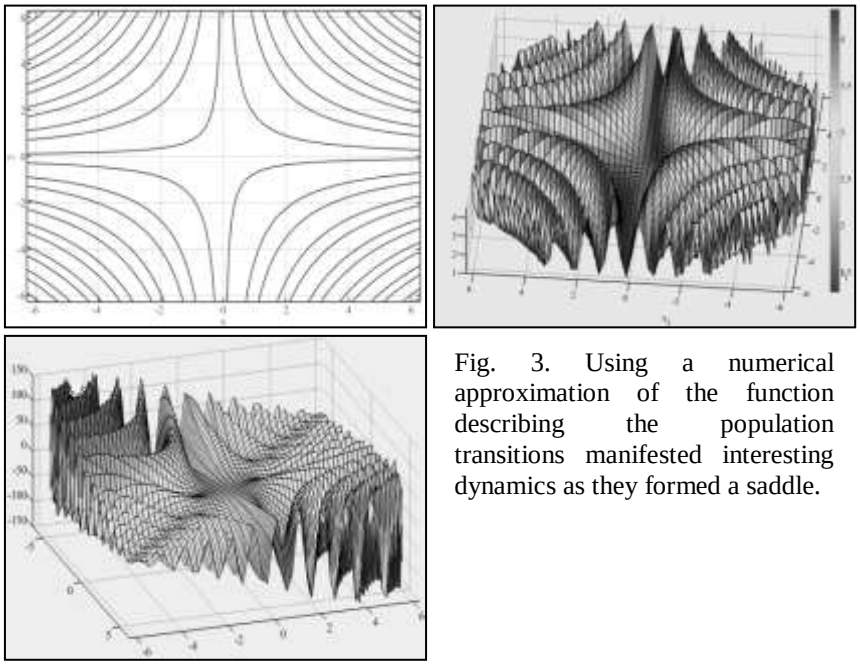

Fig. 3. Using a numerical approximation of the function describing the population transitions manifested interesting dynamics as they formed a saddle.

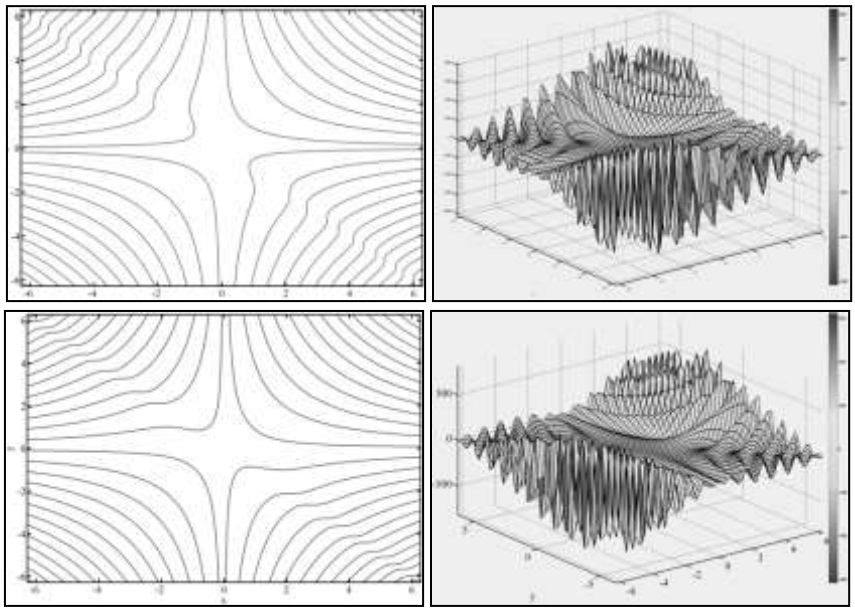

Fig. 4. Numerical representation of the first partial derivative with respect to $N_{p,y}$ (upper left and right) and with respect to $N_{p,z}$ (lower left and right).

Accordingly, as far as metabolic data are concerned, the determination of the factor *k* was implemented with numerical approximations. We have assumed again that *k* is a nonlinear factor. The first aim was to determine the dynamics of the factor *k* i.e. how it changes as a function of concentration. In order to do this, we used the simplified model presented in Figure 5. Glucose measurements were taken from cell culture supernatants (C_G). We assumed that glucose entering the cell was transformed as a total into ATPs and pyruvate. Since cells presumably follow a lactic acid fermentation cycle, pyruvate should be transformed into lactate through LDH.

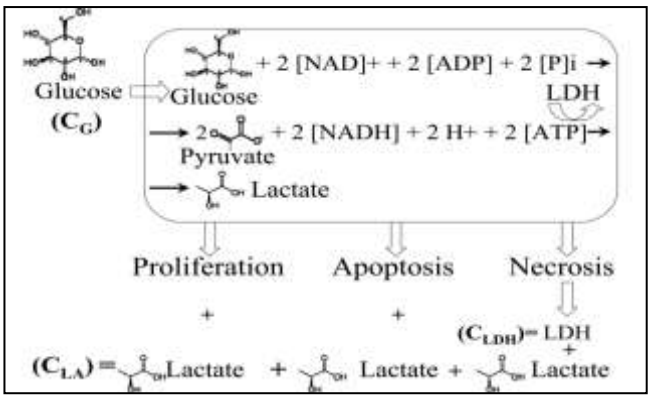

Fig. 5. A simple model of cell fate and measurements of metabolic factors.

In addition, the enzyme LDH (Lactate Dehydrogenase) was measured as a function of the total population of necrotic cells (C_{LDH}). It is important to note that LDH is released from the cells only if cell lysis takes place, thus allowing the contents of the cytosol to be released in the extracellular medium.

At the same time the measured lactate (C_{LA}) was considered to be diffused from both living and apoptotic cells and also released from necrotic cells due to cell membrane lysis. Finally, we accounted for three possible cell fates: progression of proliferation (N_v), necrosis (N_n) and apoptosis (N_{ta}). One of the first correlations calculated was that of the measured LDH and the respective number of necrotic cells. We would be expected to observe a positive correlation between the two factors. We have previously reported that LDH concentration and necrotic cell population indeed showed a positive correlation in two particular cases: untreated cells and cells treated with a large dose of prednisolone (700uM) [23]. This effect can be interpreted as follows: all other glucocorticoid concentrations beside necrotic cell death, also lead to the rupture of the cell membrane and cell lysis. Interestingly, the largest concentration that would be expected to have a lytic effect due to the overdose *per se*, showed a negative correlation, exactly matching that of cells with no glucocorticoid treatment. As mentioned earlier, we have attempted to impute numerically the factor *k* by plotting conditions at time *t+1* vs. conditions at time *t*. In other

words we have attempted to model the total cell population over time as a function of the drugs concentrations. As it is shown in Figure 6 it appeared that cells followed complicated dynamics under the influence of the glucocorticoids even when the cell populations are separated into viable, necrotic and apoptotic. The manifested oscillatory behaviour indicates that cells proliferate with nonlinear dynamics, and despite the very few data points, their behaviour could still be revealed. In addition, the plotting of the phase-space of metabolic factors shows that the transition from one state to the other also follows oscillations (Figure 7).

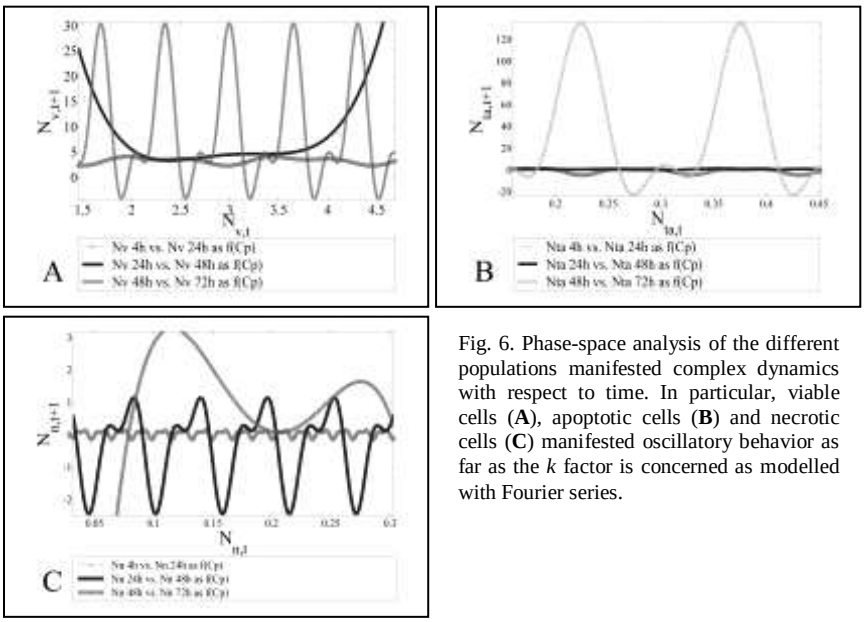

Fig. 6. Phase-space analysis of the different populations manifested complex dynamics with respect to time. In particular, viable cells (**A**), apoptotic cells (**B**) and necrotic cells (**C**) manifested oscillatory behavior as far as the *k* factor is concerned as modelled with Fourier series.

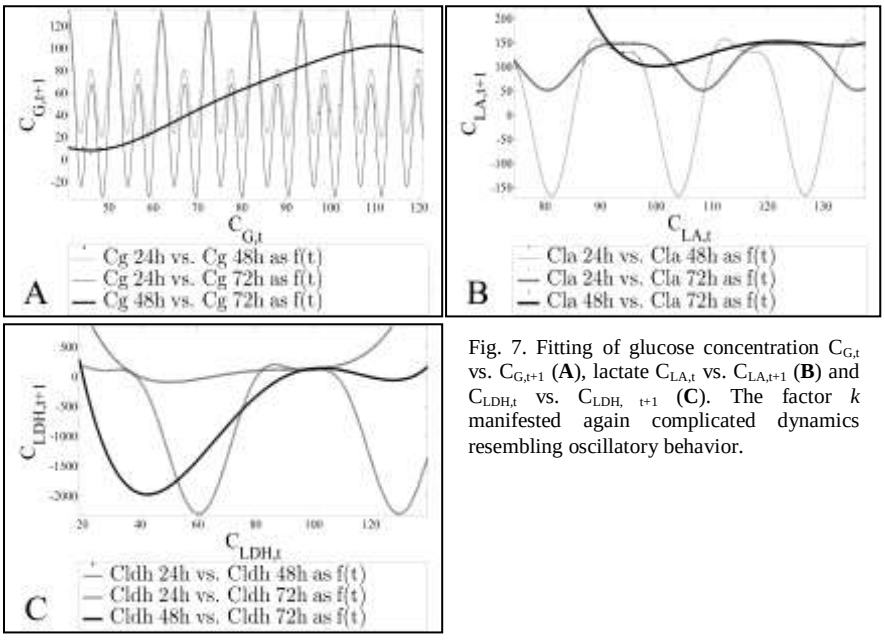

Fig. 7. Fitting of glucose concentration $C_{G,t}$ vs. $C_{G,t+1}$ (**A**), lactate $C_{LA,t}$ vs. $C_{LA,t+1}$ (**B**) and $C_{LDH,t}$ vs. $C_{LDH,\ t+1}$ (**C**). The factor *k* manifested again complicated dynamics resembling oscillatory behavior.

In the present work we attempted to identify non-linear factors of cell proliferation under the influence of chemotherapeutics, and more specifically under the influence of the glucocorticoid prednisolone. We attempted to establish an initial theoretical framework for the analysis of such phenomena and for future considerations. Cell growth appeared to be of a non-linear character. This knowledge could be proved useful in the treatment of tumors, since understanding the biology of proliferation would lead us to a better understanding of cellular resistance to chemotherapeutics. Biological systems are extremely complicated and they manifest, without doubt, non-linear/chaotic phenomena. Therefore, as we have mentioned in previous works, we believe that the maturity of biological sciences would come through integration with other disciplines, such as mathematics and physics, and the ability to give generalized models for these phenomena. Such an example is the understanding of cell proliferation in which we attempted to contribute with hints.

We also attempted to create a modelling framework, along with its mathematical formulation, for describing the dynamics of leukemic cells under the influence of glucocorticoids. We used two factors in our analysis: cell populations, including changes in viability and cell death, and metabolic factors. Approximations of experimental data of course require large datasets, in order to have a more precise view of the fitted phenomena. However, we must mention that obtaining large amounts of data from biological systems can sometimes be proved to be a tedious task. This is owed to the fact that cells in culture preserve a proliferation potential and if they remain in culture for a long period of time, the observed results should be accounted for additional effects besides the one under investigation. In the present analysis, the *Jacobian* matrix J determines the transition dynamics of the system from one state to the next. In a previous work the use of Jacobian matrices was used for the determination of the possible dynamics of a system at a metabolic state [22]. There is a great amount of mathematical formulations concerning biological systems dating back in the early 19^{th} century but the whole idea of integrating biological systems with analytical or stochastic formulations is still in its infancy [13, 19-21]. Therefore, such approaches could prove very useful in gaining more insight into the proliferation dynamics of cell populations and the dynamics emerging under the influence of external stimuli such as chemotherapeutics.

APPENDIX

The functions that have been used for the fitting of the data and the mathematical formulations were the following: Quadratic:

a) $y = ax^2 + bx + c$

b) Cubic: $y = ax^3 + bx^2 + cx + d$

c) polynomial of n^{th} *,* m^{th} *degree:*

$f(x, y) = a_n x^n b_m y^m + a_{n-1} x^{n-1} b_{m-1} y^{m-1} + \ldots + a_1 x b_1 y + a_0 b_0$ *d) d)* 1^{st} *order Fourier Series:* $a_1 \cos(xw) + b_1 \sin(xw) + a_0$

e) 2^{nd} *order Fourier Series:*

$a_2 \cos(2xw) + a_1 \cos(xw) + b_2 \sin(2xw) + b_1 \sin(xw) + a_0$ *ff) Lotka-Voltera equations:*

$$\frac{dx}{dt} = x(\alpha - \beta y)$$

$$\frac{dy}{dt} = -y(\gamma - \delta x)$$

g) Kolmogorov variation of Lotka-Voltera functions

$$\frac{dx}{dt} = f(x, y)x$$

$$\frac{dy}{dt} = g(x, y)y$$

$$f(x, y) = A_0 - A_1 x - A_2 y$$

$$g(x, y) = B_0 - B_1 x - B_2 y$$

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